THE JOURNAL OF DAIRY RESEARCH

LONDON
Cambridge University Press
FETTER LANE E.C. 4

BOMBAY · CALCUTTA · MADRAS
Macmillan

TOKYO
Maruzen Company Ltd

All rights reserved

THE JOURNAL OF DAIRY RESEARCH

EDITED FOR

The Dairy Research Committee of the Empire Marketing Board

BY

R. STENHOUSE WILLIAMS, M.B., C.M.
D.Sc., D.P.H., L.R.C.P. & S.E.
DIRECTOR OF THE NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING,
UNIVERSITY OF READING

OVERSEA CORRESPONDENTS

J. H. GRISDALE, D.Sc.A., D.Agr.
Deputy Minister of Agriculture
for the
Dominion of Canada

Prof. H. B. DAVEL, B.Sc.
Director of the Dairy Research Institute
Transvaal University College,
Pretoria, South Africa

W. RIDDET, B.Sc., N.D.A., N.D.D.
Director of the Dairy Research Institute and
Dean of Animal Husbandry at the Massey
Agricultural College, New Zealand

Prof. CONNELL BOYLE, M.A., B.Sc.,
D.I.C., Ph.D.
Dean of the Faculty of Dairy Research,
University College, Cork

Col. A. OLVER, C.B., C.M.G., F.R.C.V.S.
Animal Husbandry Expert, Imperial Council of
Agricultural Research, India

VOLUME III

CAMBRIDGE
AT THE UNIVERSITY PRESS
1932

PRINTED IN GREAT BRITAIN

Jour. of Dairy Research

ERRATA. Vol. III, No. 1.

Page 114, line 8, *for* $2{\cdot}25 \times 0{\cdot}75\,\mu$ *read* $2{\cdot}25 - 6 \times 0{\cdot}75\,\mu$.

„ line 18, *for* $2{\cdot}25 \times 1{\cdot}25\,\mu$ *read* $2{\cdot}25 - 3 \times 1{\cdot}25\,\mu$.

Page 146, line 5, *for* faeces *read* factors.

VOLUME III DECEMBER, 1931 No. 1

25. THE MINERAL CONSTITUENTS AND CITRIC ACID CONTENT OF MILK

BY L. A. ALLEN, M.SC., PH.D.
Hannah Dairy Research Institute.

(With 9 Figures.)

(*THE material for this paper was collected in the course of the review of the literature concerning the condensing and drying of milk and milk by-products which is now being prepared by the Hannah Dairy Research Institute with the aid of a grant from the Empire Marketing Board.*)

Though the mineral constituents normally represent less than 1 per cent. of cow's milk their importance in many dairying problems is well realised. For example, the calcium-magnesium-phosphate-citrate balance is a most important factor in connection with coagulation by heat and in the alcohol test, even minute alterations in the balance producing a very marked effect.

It may safely be said that sufficient experimental data are available to demonstrate the influence of the mineral constituents. Unfortunately, however, much of the data is confusing, some of it is contradictory and a great deal of it has been compiled with too little regard to the possible experimental error. Results in detail for the complete analysis of a sample of milk have been given by extremely few authors and no one so far appears to have collected together the results that are available, either for the complete analysis or for the separate constituents, so as to provide figures for the maximum, minimum and mean percentages, or give an accurate idea of the range of variation.

In view, therefore, of the importance of the subject and of the desirability of readily available, comprehensive data—the lack of which has on several occasions apparently led to incorrect conclusions in feeding experiments, etc. —there is summarised in this paper the present state of knowledge as regards the quantities of the various mineral constituents in milk, the extent and causes of their variation and, as far as possible, the partition of each in the various phases. Citric acid, since it exerts an influence comparable in many respects with that of the inorganic constituents, is included in the survey, but occupies a separate section.

THE NATURE AND STATE OF THE MINERAL CONSTITUENTS IN MILK.

By the mineral constituents of milk we mean those which contain only inorganic elements in their molecules or ions. Of such elements the following have been shown to be present: potassium, sodium, calcium, magnesium, chlorine, phosphorus, sulphur, and in smaller quantities iron, copper, zinc,

aluminium, manganese and iodine, with traces of silicon, boron, titanium, vanadium, rubidium, lithium and strontium.

It must always be remembered that milk is a heterogeneous system, but if we leave out for the moment the question of the adsorption of the inorganic milk constituents on the surfaces of the colloidal phase we may discuss the state of these constituents.

Milk constituents may be divided into three classes: (1) those wholly in solution, (2) those partly in solution and partly in suspension, (3) those wholly in suspension.

If the solid phase be removed by ultrafiltration methods the distribution of the inorganic constituents between the phases may be determined. In this way it has been shown that all the sodium, potassium and chlorine are in true solution, while the calcium, magnesium and phosphorus are partly in solution and partly in suspension.

Attempts have been made to postulate a series of salt combinations for those constituents in true solution, *i.e.* to partition the sodium as sodium chloride, phosphate, etc., and so on for each element. While it may be admitted that all the chlorine exists probably as chlorine ions, the sulphur as sulphate ions and the phosphorus as phosphate ions and undissociated phosphate, it must be realised that attempts to assign to these anions corresponding cations betray a distressing ignorance of the fact that we have to deal with a polyionic system. Our present knowledge of such systems is far from enabling us to deal with the question of salt combination. Not only does the presence of ionic equilibria render any classification from the molecular standpoint abortive but milk being a heterogeneous system the intervention of a solid phase further complicates the problem. We can, however, discuss with profit the distribution of the inorganic constituents between the two phases.

Partition of the inorganic constituents in milk.

Investigations in this subject have been comparatively few, but it is possible by careful consideration of the work which has been done to arrive at some conclusion as to the condition of the soluble and insoluble salts of milk and their relation to the colloidal particles of protein. It is scarcely necessary to add, perhaps, that a great deal more work will be essential before that conclusion becomes in any way comprehensive. Most of the data obtained so far relate to the partition of the calcium and phosphorus.

Van Slyke and Bosworth (62, 63) attacked the question of the condition of casein and salts in milk and the effect on them of the process of souring. Their first series of experiments embodied four lines of investigation: (1) the properties and composition of milk serum or, in other words, the substances in solution, (2) a similar investigation of the insoluble constituents, (3) the relative acidities of milk and its serum, (4) the condition of the salts in milk.

The serum was obtained by filtration of the milk through a Pasteur-Chamberland filter candle under a pressure of 40–45 lb. per square inch (using

the apparatus described by Briggs and Lapham(64)), chloroform being added to prevent bacterial decomposition. The first 50–75 c.c. in each filtration were discarded in accordance with the observation of Rupp(65) that, until this amount has passed through, the filter adsorbs some of the constituents. Results of analyses of two samples of milk and its serum are shown in Table I. Details of analysis are, unfortunately, not given by these authors. Moreover the figures for six constituents are for one sample only.

It will be noticed that some of the constituents are present in the serum only, some only in the insoluble residue, and some in both serum and residue. We may therefore divide the constituents of milk into three classes: (1) those in true solution in the serum—lactose, citric acid, potassium, sodium, and non-protein nitrogen, (2) those partly in solution and partly in colloidal suspension

Table I. *Constituents of milk and milk serum (Van Slyke and Bosworth).*

	Sample 1			Sample 2		
Constituents	Milk (100 ml.)	Serum (100 ml.)	Constituents in serum (%)	Milk (100 ml.)	Serum (100 ml.)	Constituents in serum (%)
Sugar	—	—	—	5·75	5·75	100·00
Casein	3·35	0·00	0·00	3·07	0·00	0·00
Albumin	0·525	0·369	70·29	0·506	0·188	37·15
N in other compounds	—	—	—	0·049	0·049	100·00
Citric acid	—	—	—	0·237	0·237	100·00
P (organic and inorganic)	0·125	0·067	53·60	—	—	—
P (organic)	0·096	0·067	70·00	0·087	0·056	64·40
Ca	0·128	0·045	35·16	0·144	0·048	33·33
Mg	0·012	0·019	75·00	0·013	0·007	53·85
K	0·354	0·352	99·44	0·120	0·124	100·00
Na				0·055	0·057	100·00
Cl	0·081	0·082	100·00	0·076	0·081	100·00
Ash	—	—	—	0·725	0·400	55·17

—albumin, inorganic phosphate, calcium and magnesium, (3) those entirely in colloidal suspension—fat and casein.

It was found that the insoluble portion remaining on the outside of the filter readily formed a suspension in water and even when freed from soluble substances by repeated suspension and filtration this remained permanent as long as it contained a preservative. Reference to Table I shows that all the citric acid was found in the serum, so that this substance cannot very well be the agency which is responsible for holding the particles of protein in suspension—a theory which was previously maintained.

In order to investigate the relations existing between casein and the inorganic constituents of milk detailed analyses were made of sixteen samples from thirteen individual cows and values for the following obtained: (*a*) casein, (*b*) total phosphorus, (*c*) soluble phosphorus, (*d*) insoluble phosphorus (*i.e.* $b - c$), (*e*) insoluble organic phosphorus (casein $\times$ 0·0071), (*f*) insoluble inorganic phosphorus ($d - e$), (*g*) total calcium, (*h*) soluble calcium, (*i*) insoluble

calcium ($g - h$), (j) total magnesium, (k) soluble magnesium, (l) insoluble magnesium ($j - k$).

The results of these analyses for protein, casein, and phosphorus are shown in Table II, and those for soluble and insoluble calcium and magnesium in Table III, while Table IV shows the values for soluble calcium, magnesium and

Table II. *Percentages of protein, casein, and phosphorus in milk (Van Slyke and Bosworth).*

Cow No.	Stage of lactation	Total protein	Casein	Phosphorus					Ratio Org. P / Insol. Inorg. P
				Total	Soluble	Insoluble			
						Total	Organic in casein	Inorganic (phosphate)	
1	3 days	4·35	3·48	0·1272	0·0818	0·0454	0·0247	0·0207	1 : 0·83
2	1 month	3·31	2·73	0·1150	0·0595	0·0555	0·0194	0·0361	1 : 1·86
3	1 „	3·53	2·78	0·1010	0·0494	0·0516	0·0197	0·0319	1 : 1·62
3	11 months	4·91	4·09	0·1111	0·0536	0·0575	0·0290	0·0285	1 : 0·98
4	3 „	3·93	3·09	0·1278	0·0563	0·0715	0·0219	0·0496	1 : 2·26
5	3 „	3·45	2·88	0·0943	0·0475	0·0468	0·0204	0·0264	1 : 1·29
5	7 „	3·45	2·70	0·0870	0·0334	0·0536	0·0192	0·0344	1 : 1·79
6	5 „	4·05	2·92	0·1008	0·0356	0·0652	0·0207	0·0445	1 : 2·15
7	6 „	4·07	3·40	0·1063	0·0548	0·0515	0·0241	0·0274	1 : 1·14
7	10 „	4·80	3·56	0·1010	0·0340	0·0670	0·0253	0·0417	1 : 1·65
8	7 „	4·39	3·58	0·1157	0·0550	0·0607	0·0254	0·0353	1 : 1·39
9	8 „	4·33	3·47	0·1036	0·0364	0·0672	0·0246	0·0426	1 : 1·73
10	9 „	3·65	3·10	0·1097	0·0610	0·0487	0·0220	0·0267	1 : 1·22
11	10 „	4·17	3·36	0·1090	0·0434	0·0656	0·0239	0·0417	1 : 1·74
12	11 „	4·35	3·14	0·1060	0·0286	0·0774	0·0223	0·0551	1 : 2·47
13	12 „	5·71	4·97	0·1310	0·0442	0·0868	0·0353	0·0515	1 : 1·46

Table III. *Percentages of soluble and insoluble* Ca *and* Mg *in milk (Van Slyke and Bosworth).*

Cow No.	Stage of lactation	Calcium			Magnesium		
		Total	Soluble	Insoluble	Total	Soluble	Insoluble
1	3 days	0·1607	0·0734	0·0873	0·0156	0·0142	0·0013
2	1 month	0·1381	0·0511	0·0870	0·0136	0·0117	0·0019
3	1 „	0·1362	0·0544	0·0818	0·0180	0·0142	0·0038
3	11 months	0·1559	0·0534	0·1025	0·0170	0·0156	0·0014
4	3 „	0·1483	0·0343	0·1140	0·0184	0·0128	0·0056
5	3 „	0·1396	0·0531	0·0865	0·0156	0·0124	0·0032
5	7 „	0·1256	0·0454	0·0802	0·0147	0·0134	0·0013
6	5 „	0·1413	0·0373	0·1040	0·0160	0·0127	0·0033
7	6 „	0·1464	0·0526	0·0938	0·0144	0·0121	0·0023
7	10 „	0·1523	0·0450	0·1073	0·0177	0·0127	0·0050
8	7 „	0·1506	0·0439	0·1062	0·0153	0·0118	0·0035
9	8 „	0·1503	0·0440	0·1063	0·0171	0·0126	0·0045
10	9 „	0·1410	0·0543	0·0867	0·0168	0·0141	0·0027
11	10 „	0·1379	0·0357	0·1022	0·0168	0·0119	0·0049
12	11 „	0·1659	0·0414	0·1245	0·0191	0·0123	0·0068
13	12 „	0·2167	0·0669	0·1498	0·0236	0·0163	0·0073

phosphorus expressed as the percentage of the total for each constituent respectively. It is interesting to note that differences in total phosphorus are not due entirely to the differences in casein, but that the soluble phosphorus and the insoluble inorganic phosphorus both fluctuate considerably. Owing to the rather wide variation in the values found by different investigators for the phosphorus content of casein the value 0·0071, used (directly) by Van Slyke

and Bosworth in calculating the insoluble organic phosphorus and (indirectly) in calculating the insoluble inorganic phosphorus, is open to question. In consequence the values for the ratio "organic P/insoluble inorganic P" may actually be rather different from those shown in the table. However, this possible error would be comparatively small, so that in any case their conclusion that this ratio shows wide variations remains valid. From the figures shown it may be seen that this ratio varies from 1 : 0·83 to 1 : 2·47. Now if the inorganic phosphates are in combination with the casein this ratio should be fairly constant, so we are justified in assuming that there is no such chemical combination. In view of the fact that it has been held by other investigators that part of the calcium phosphate and the protein are chemically combined this is a very important result and it is unfortunate that where such conclusions rest on the accuracy of the methods of analysis employed no details of these are supplied.

Table IV. *Soluble* Ca, Mg, *and* P *expressed as percentages of total* Ca, Mg, *and* P *respectively* (*calculated from figures given by Van Slyke and Bosworth*).

Cow No.	Stage of lactation	Soluble Ca as percentage of total Ca	Soluble Mg as percentage of total Mg	Soluble P as percentage of total P
1	3 days	45·69	91·04	64·31
2	1 month	37·00	86·03	51·73
3	1 ,,	39·95	78·89	48·91
3	11 months	34·25	91·78	48·26
4	3 ,,	23·13	69·56	44·04
5	3 ,,	38·03	79·49	50·35
5	7 ,,	36·15	91·16	38·39
6	5 ,,	26·40	79·39	35·33
7	6 ,,	35·93	84·01	51·57
7	10 ,,	29·55	71·75	33·65
8	7 ,,	29·15	77·11	47·54
9	8 ,,	29·28	73·69	35·14
10	9 ,,	38·51	83·91	55·54
11	10 ,,	25·89	70·83	39·82
12	11 ,,	24·95	64·40	26·98
13	12 ,,	30·86	69·08	33·74

The results in Tables III and IV indicate that there are considerable differences between individual cows in the calcium and magnesium contents of the milk, both as regards the total quantities present and also for those portions present in true solution. Thus total calcium varies from 0·1256 to 0·2167 per cent. and soluble calcium from 0·0343 to 0·0734 per cent., while total magnesium varies from 0·0136 to 0·0236 per cent. and soluble magnesium from 0·0117 to 0·0163 per cent. In each case one cow is responsible for all the low values and another cow for all the high values. Reference to the table shows that the first cow was in an early stage of lactation while the second was at the end of lactation, so the differences are probably due partly to this factor. It will be noticed that the greater part of the calcium is present in the insoluble condition, while only a trace of the magnesium is insoluble. However, owing to the fact that the amount of total calcium is considerably greater than the amount of total magnesium the quantity of calcium in solution is still far in excess of the quantity of soluble magnesium.

Van Slyke and Bosworth next attacked the question of the form in which the insoluble calcium and phosphorus are present in milk and were led, by an ingenious series of steps, to the conclusion that the insoluble inorganic phosphate of milk is neutral calcium phosphate ($CaHPO_4$). Thus from the results of the analyses in Tables II and III they calculated the amounts of the acids and bases present as gram equivalents: (1) casein as a gram equivalent of octovalent casein (assuming that this substance combines with eight equivalents of calcium), (2) insoluble inorganic phosphate as gram equivalents of dibasic acid, (3) the sum of the gram equivalents of casein and phosphates, (4) insoluble calcium as gram equivalents, (5) insoluble magnesium as gram equivalents, (6) the sum of insoluble calcium and magnesium as gram equivalents.

On comparing the values for (3) and (6) it was seen that there was close agreement between them, which means that the quantitative relation between the bases (Ca and Mg) and the acids (casein and phosphoric acid) is that required theoretically to give dibasic calcium phosphate with a trace of dibasic magnesium phosphate and the calcium caseinate which contains eight equivalents of calcium. On the other hand they point out that the same figures might be used to show that the compounds are present as acid caseinate and tricalcium and trimagnesium phosphates.

In order, then, to decide between the two alternatives thus presented a partial separation of casein and insoluble inorganic phosphate was made. This was effected by centrifuging milk for different times and at different speeds. The separator slime obtained in each case was analysed after first triturating with 95 per cent. alcohol and drying the precipitate. It was found that, although the inorganic phosphorus was unevenly distributed in the different deposits, there was the same balance between the bases (Ca and Mg) and the acids (casein and phosphoric acid). Now this could only be the case if the inorganic phosphorus were present as neutral calcium phosphate ($CaHPO_4$), for otherwise the balance between the bases and acids would be altered by an alteration in the relative amount of inorganic phosphate present—$Ca(H_2PO_4)_2$ giving an excess of acid and $Ca_3(PO_4)_2$ giving an excess of base. The experimental evidence thus points to the fact that the insoluble inorganic phosphate of milk is neutral calcium phosphate.

Further evidence of the impossibility of a chemical combination existing between the casein and the insoluble phosphate was obtained by determining the ratio inorganic P/organic P in the separator slime obtained in each case. It was found to exhibit considerable variations, as in the previous experiment.

As regards acidity Van Slyke and Bosworth found that the serum obtained by ultrafiltration reacted slightly acid and showed the same titratable acidity as the original milk. The acidity (as ml. of decinormal alkali per 100 ml.) of milk and its serum for eight samples of milk was found to be as follows:

No. of sample	1	2	3	4	5	6	7	8
Milk	4·8	6·2	4·2	6·0	6·4	4·4	7·0	6·6
Serum	5·0	6·2	4·2	5·8	6·4	4·4	6·8	6·4

In the second paper Van Slyke and Bosworth describe experiments carried out to determine the effect of bacterial souring on the soluble and insoluble constituents of milk. Fresh milk and the serum obtained from it by filtration through a porous porcelain filter were first analysed in detail. Another portion of the same milk was then allowed to sour at room temperature by the action of a mixture of *S. lactis* and *B. lactis aerogenes* and similar analyses made when the milk was curdled. Their results are given in Table V, and show that all those inorganic constituents which were originally insoluble have been rendered soluble as the result of the developed acidity. Thus soluble calcium increases from 33·33 to 100 per cent., soluble magnesium from 53·85 to 100 per cent., and soluble phosphorus from 64·4 to 100 per cent.

Table V. *Effect of souring on the soluble and insoluble constituents of milk (Van Slyke and Bosworth).*

		Serum from fresh milk		Serum from sour milk	
Constituents	Original milk (100 ml.)	Serum (100 ml.)	Milk constituents in serum (%)	Serum (100 ml.)	Milk constituents in serum (%)
Sugar	5·75	5·75	100·00	4·48	—
Casein	3·07	0·00	0·00	0·00	0·00
Albumin	0·506	0·188	37·15	0·506	100·00
N in other compounds	0·049	0·049	100·00	0·049	100·00
Citric acid	0·237	0·237	100·00	0·000	0·00
P (inorganic)	0·087	0·056	64·40	0·090	100·00
Ca	0·144	0·048	33·33	0·148	100·00
Mg	0·013	0·007	53·85	0·014	100·00
K	0·120	0·124	100·00	0·120	100·00
Na	0·055	0·057	100·00	0·058	100·00
Cl	0·076	0·081	100·00	0·079	100·00
Ash	0·725	0·400	55·17	0·690	95·17
Lactic acid	0·000	0·000	0·00	1·124	—

The same authors next determined the extent to which calcium and phosphorus were dissolved at different stages in the process of souring. Fresh milk was inoculated with *S. lactis* and incubated at 32·2° C. At frequent intervals up to 25 hours, samples were analysed for casein, soluble and insoluble calcium, and soluble and insoluble phosphorus. Assuming that insoluble phosphate was present as $CaHPO_4$ and that calcium was combined with casein as tetracalcium caseinate (an assumption which was justified since these authors had previously shown this to be the case), the amount of calcium dissolved at each stage in the case of each of these compounds was calculated. The results are shown in Table VI. The results are of special interest in showing the extent to which insoluble calcium and phosphorus become soluble in the early stages of souring. Thus, according to these authors, after 4 hours, 8·7 per cent. of the calcium and phosphorus originally present as $CaHPO_4$ is dissolved, after 8 hours 27·4 per cent., and after 13½ hours, 100 per cent. The calcium combined with the casein, on the other hand, is acted upon more slowly, for after 5 hours none had been dissolved, while it required 25 hours to dissolve the whole of it.

Similar analyses were made for magnesium and it was shown that this substance went into solution completely in 11½ hours under the same conditions.

Although Van Slyke and Bosworth have shown that under the conditions normally prevailing in fresh milk insoluble calcium is present as neutral calcium phosphate and probably tetracalcium caseinate it must be borne in mind that combination between calcium and phosphoric acid and between calcium and casein is in both cases a function of *p*H. Thus reference to the titration curves of casein and paracasein with calcium hydroxide, given by Palmer and Richardson (66) and reproduced in Fig. 1, shows that at the isoelectric point of casein—*p*H 4·62—the amount of calcium which combines with this substance is nil. It is for this reason that development of acidity in milk brings more and more of the insoluble calcium into solution until, when the *p*H reaches 4·62, the whole of the calcium is soluble. The effect of acidity in increasing the solubility of this substance was, in fact, demonstrated by Van Slyke and Bosworth in the experiments previously described, though they did not explain their results in terms of *p*H.

Table VI. *Percentages of soluble and insoluble calcium and phosphorus in milk at different stages of souring (Van Slyke and Bosworth).*

Age of milk (hours)	Insoluble Ca in 100 ml. Total (g.)	As $CaHPO_4$ (g.)	As caseinate (g.)	Insoluble Ca dissolved in case of $CaHPO_4$ (%)	caseinate (%)	Insoluble inorganic P in 100 ml. (g.)	Insoluble P dissolved (%)
Fresh	0·1144	0·0515	0·0629	—	—	0·0399	—
4	0·1102	0·0470	0·0632	8·7	0·0	0·0364	8·7
5	0·1090	0·0457	0·0633	11·2	0·0	0·0354	11·2
6	0·1041	0·0431	0·0610	16·3	3·2	0·0334	16·3
7	0·0974	0·0418	0·0556	18·8	11·7	0·0324	18·8
8	0·0922	0·0374	0·0548	27·4	13·0	0·0290	27·4
9	0·0775	0·0319	0·0456	38·0	27·6	0·0247	38·0
10	0·0648	0·0255	0·0393	50·5	37·6	0·0198	50·5
11	0·0560	0·0180	0·0380	65·0	39·7	0·0140	65·0
12	0·0368	0·0049	0·0319	90·5	—	0·0038	90·5
13	0·0191	0·0019	0·0172	96·3	72·7	0·0015	96·3
13½	0·0154	0·0000	0·0154	100·0	75·5	0·0000	100·0
15	0·0092	—	0·0092	—	85·4	—	—
19	0·0088	—	0·0088	—	86·0	—	—
25	0·0000	—	0·0000	—	100·0	—	—

György (67) also found that the amounts of calcium and phosphorus in solution increase as the *p*H is lowered and that at the isoelectric point of casein no calcium remains combined with the protein.

For this reason the amounts of calcium and phosphorus in the whey from milk coagulated by development of spontaneous souring are considerably higher than those in the whey from a coagulum produced by the action of rennet at constant *p*H.

György next determined the relation between diffusible and indiffusible inorganic phosphorus in milk by means of ultrafiltration experiments with de Hain filter membranes. Table VII shows the results of his determinations,

Table VII. *Diffusible and indiffusible phosphorus in milk (György).*

Experiment	Inorganic P—mg. per 100 c.c. of milk 1	2	3	4	P as percentage of total inorganic P 1	2	3	4
Whole milk	33·3	35·8	29·5	30·9	—	—	—	—
Ultrafiltrate	13·3	19·0	13·3	12·7	39·8	53·1	45·3	41·6

and it would appear from these that 40–55 per cent. of the inorganic phosphorus in milk is in the diffusible form and 45–60 per cent. in the indiffusible form. Van Slyke and Bosworth, it will be remembered, found a somewhat lower value (30–45 per cent.) for the insoluble inorganic phosphorus by filtration through a Pasteur-Chamberland filter.

The same author also found that tryptic hydrolysis at constant *p*H of the casein in milk resulted in a consistent increase in the amounts of dialysable calcium and phosphorus. He concluded from this that a part of the dialysable calcium and phosphorus is ordinarily adsorbed by the casein particles and that

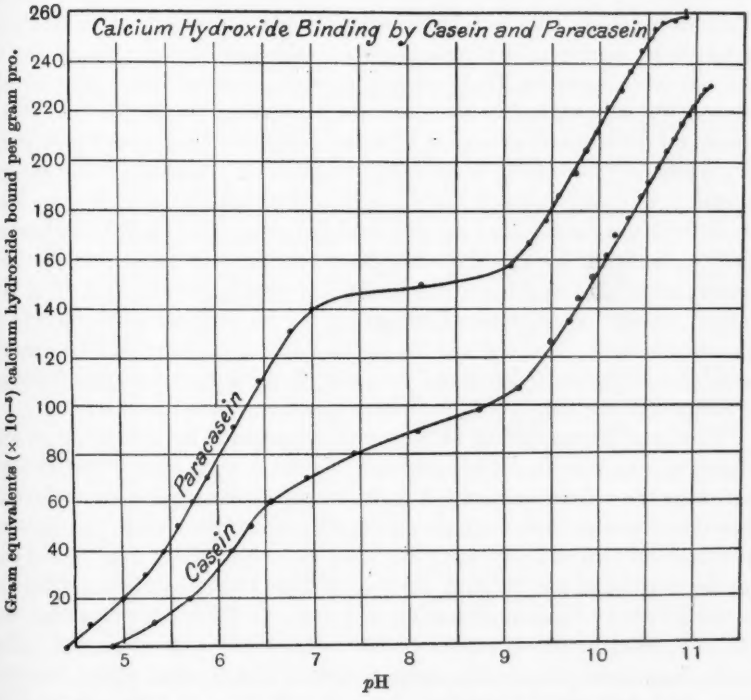


Fig. 1. Titration curves of casein and paracasein (Palmer and Richardson).

tryptic degradation of the protein merely liberates it into solution. If, as he points out, it were insoluble calcium phosphate which was held in suspension by the casein particles then hydrolysis of the latter at constant *p*H would merely lead to a precipitation of the calcium phosphate. It may be mentioned that the data given by György show considerably lower values for dialysable calcium and phosphorus obtained from milk treated with boiled trypsin than from the untreated milk, and no explanation of this apparent anomaly is offered by the author.

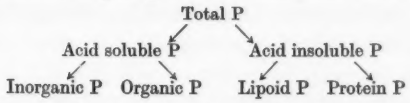
The conclusions of Van Slyke and Bosworth on the one hand and of György

on the other appear at first to be rather conflicting, for whereas the former authors demonstrate the presence of insoluble calcium phosphate in the milk the latter argues that since degradation of the protein liberates soluble calcium and phosphate it cannot be the insoluble salt which is held in suspension by the casein. However, it must be remembered that there are a number of basic and acidic radicles present in the milk, both in the soluble and insoluble phases, and provided the laws relating to the respective solubility products are satisfied it is possible for insoluble salts to remain in equilibrium with ions in solution. Or it may be that in milk the various radicles have not arrived at equilibrium but are merely in the early stage of a slow process. Indeed the fact that the effect of heat on milk is to precipitate insoluble calcium, phosphate and citrate is an indication that milk is not in stable equilibrium with respect to the various components. To discuss this question in detail has no place at this phase of the subject, and in fact there are, in any case, too few data to afford a basis for definite conclusions, but the main object at the moment is to point to the possibility of soluble calcium salts and soluble phosphates being present in milk at the same time as insoluble calcium phosphate. Thus while there is no reason to doubt the fact that the only insoluble phosphate in milk is the neutral $CaHPO_4$, as found by Van Slyke and Bosworth, and that there is no chemical combination between this and the casein it is equally possible that there is a certain amount of adsorption between the soluble calcium salts and soluble phosphates on the one hand and the casein particles on the other which would result in a liberation of soluble calcium and phosphorus after tryptic digestion of the casein at constant *p*H, as György appears to have discovered.

The phosphorus content of milk was investigated by Jordan, Hart and Patten (6), who separated it into (*a*) total, (*b*) soluble, (*c*) inorganic. The methods used were those given by Hart and Andrews (100): (1) Total phosphorus was estimated by Neumann's (41) method. (2) For determinations of soluble phosphorus hydrochloric acid (0·2 per cent.) was used as an extracting reagent and also for the purpose of precipitating the proteins, the phosphorus being estimated in the filtrate by Neumann's method as before. (3) Inorganic phosphorus was also estimated in the filtrate from the hydrochloric acid extraction. After neutralisation to litmus with ammonia 10 g. NH_4NO_3 were added for every 200 g. of filtrate and the solution warmed to 65° C. 2 c.c. HNO_3 (sp. gr. 1·20) and 25 c.c. neutral ammonium molybdate solution were now added, the mixture stood at 65° C. for 15 minutes and was then filtered. The phosphorus in the precipitate was estimated as magnesium pyrophosphate. Table VIII shows the results obtained by Jordan, Hart and Patten by the above method for the milk of two Holstein cows.

It is to be noted that the inorganic phosphorus, estimated by this method, includes both the soluble and insoluble phosphates of the milk, since it has already been demonstrated that all the inorganic phosphate comes into solution at the isoelectric point of casein. According to these results approximately 65–80 per cent. of the total phosphorus in milk is present in the inorganic form.

Lenstrup (8) divided the phosphorus of milk into the following classes:



This division was made by using the methods of Greenwald (68), Feigl (69) and Iversen (70). Thus acid-soluble and acid-insoluble phosphorus were separated by using picric acid as a precipitant. Inorganic phosphorus was precipitated from the filtrate as ammonium magnesium phosphate and organic phosphorus determined in the residual filtrate. Phosphorus was estimated in each division by the Gregerson (71) modification of Neumann's method as

Table VIII. *Total soluble and inorganic phosphorus in milk (Jordan, Hart, and Patten).*

	Cow No. 1: Phosphorus (%)				Cow No. 2: Phosphorus (%)		
Date	Total	Soluble	Inorganic	Date	Total	Soluble	Inorganic
May 9	0·082	0·071	0·063	Dec. 30	0·102	0·083	0·072
10	0·078	0·068	0·060	31	0·104	0·084	0·071
11	0·083	0·066	0·056	Jan. 1	0·103	0·083	0·065
12	0·076	0·065	0·059	2	0·102	0·080	0·071
13	0·083	0·065	0·061	3	0·100	0·084	0·069
14	0·083	0·070	0·065	4	0·103	0·081	0·067
15	0·084	0·064	0·062	5	0·104	0·081	0·068
16	0·083	0·067	0·065	6	0·109	0·086	0·071
17	0·084	0·066	0·063	7	0·106	0·084	0·074
18	0·086	0·068	0·066	8	0·104	0·085	0·074
19	0·079	0·070	0·063	9	0·104	0·086	0·078
20	0·080	0·066	0·065	10	0·104	0·083	0·078
21	0·093	0·067	0·065	11	0·104	0·082	0·069
22	0·094	0·071	0·069	12	0·107	0·087	0·073
23	0·091	0·074	0·072				
24	0·092	0·075	0·072				
25	0·093	0·074	0·074				
26	0·093	0·071	0·073				
27	0·090	0·070	0·070				
28	0·090	0·077	0·072				
29	0·091	0·072	0·076				
30	0·089	0·070	0·072				
31	0·092	0·073	0·076				
June 1	0·092	0·072	0·060				
2	0·079	0·075	0·065				

adapted for micro-determinations by Iversen (*loc. cit.*). Lenstrup states that the method has proved capable of determining from 0·1 to 0·5 mg. of phosphorus with an error of less than 4 per cent. of the amount present. Table IX shows his results for 15 analyses of mixed milk from 30 cows (which were kept normal and healthy) over a period of 4 months.

It is to be noted that approximately five-sixths of the total phosphorus is precipitated as ammonium magnesium phosphate and therefore may be considered as inorganic.

Weekly examinations of the milk from the same herd were also made by Lenstrup over a period of 14 months. Curves showing the variation in total P, acid-soluble P and acid-soluble organic P are given in Fig. 2. It will be seen that acid-soluble organic phosphorus remains practically constant throughout

the year, while the acid-soluble inorganic phosphorus (represented by the difference between the two lower curves) is distinctly higher from September until May. The acid-insoluble or casein phosphorus (represented by the differ-

Table IX. *Partition of phosphorus in milk (Lenstrup).* *Mg.* P *per* 100 *ml.*

Date	Total P	Acid-insoluble P	Acid-insoluble P		
			Total	Inorganic	Organic
Nov. 2	96·0	16·0	80·0	69·5	10·5
9	89·7	14·2	75·5	64·4	11·1
16	95·8	15·8	80·0	67·8	12·2
23	96·4	17·1	79·3	67·5	11·8
Dec. 1	95·3	19·0	76·3	66·4	9·9
7	98·2	17·1	81·1	69·6	11·5
14	96·7	17·4	79·3	68·6	10·7
21	92·3	14·8	77·5	67·1	10·4
28	95·3	17·2	78·1	68·2	9·9
Jan. 4	96·0	18·5	77·5	66·8	10·7
14	96·7	17·4	79·3	66·8	12·5
19	93·8	15·1	78·7	67·9	10·8
27	95·3	17·8	77·5	66·5	11·0
Feb. 2	96·7	18·6	78·1	66·1	12·0
8	98·9	22·6	76·3	64·3	12·0

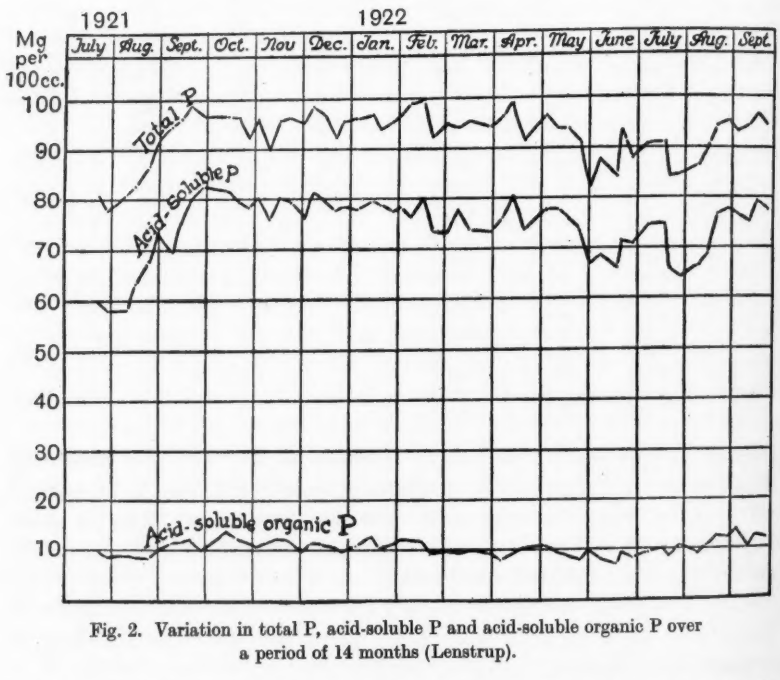


Fig. 2. Variation in total P, acid-soluble P and acid-soluble organic P over a period of 14 months (Lenstrup).

ence between the two upper curves) is also constant. We see then that according to these results the only division of the phosphorus which undergoes seasonal fluctuation is the acid-soluble inorganic phosphorus—an important deduction.

Lenstrup showed experimentally that the picric acid solution used for precipitating acid-insoluble phosphorus compounds did not cause any decomposition of the acid-soluble organic phosphorus. Also practically all the acid-insoluble phosphorus is that of casein, for determinations of the casein phosphorus after precipitation with rennet and of the phosphorus in the picric acid precipitate proved them to be almost identical.

Wardlaw (72) found that the diffusible phosphorus of cow's milk amounts to 35–55 per cent. of the total quantity present, while diffusible calcium amounts to 30–40 per cent. of the total. He stated that when a large volume of milk is dialysed against a small volume of water the freezing point of the dialysate after 24 hours approximates to that of milk and does not change as the dialysis is continued, a definite condition of equilibrium having been obtained.

Table X. *Total and diffusible calcium and phosphorus in cow's milk (Mattick).*

Total Ca (%)	Diffusible Ca (%)	Diffusible as percentage of total Ca	Total P (%)	Diffusible P (%)	Diffusible as percentage of total P
0·116	0·0319	27·50	0·099	0·038	38·38
0·126	0·0326	25·87	0·109	0·038	34·86
0·127	0·0343	27·01	0·114	0·036	31·58
0·123	0·0320	26·02	0·111	0·039	35·14
0·122	0·0295	24·18	0·104	0·030	28·85
0·119	0·0334	28·07	0·103	0·033	32·04
0·126	0·0332	26·35	0·113	0·037	32·74
0·125	0·0383	30·64	0·105	0·037	35·24
0·134	0·0328	24·48	0·109	0·034	31·19
0·130	0·0329	25·31	0·108	0·031	28·70
0·126	0·0376	29·76	0·110	0·037	33·64

Mattick (73) determined the quantity of calcium and phosphorus which diffused from milk through a parchment membrane into a 3 per cent. solution of sodium chloride in a given time. Thus 25 c.c. of milk were placed in a parchment diffusion capsule and floated in 150 c.c. of the 3 per cent. saline solution, diffusion being allowed to go on for 18 hours. At the end of that time the amounts of calcium and phosphorus which had diffused into the salt solution were estimated. This method seems to have given satisfactory results, but the values obtained were of course arbitrary and not absolute values, since an arbitrary time factor (18 hours) was used. The results obtained are reproduced in Table X. This method was actually used for the purpose of estimating the effect of heat on diffusible calcium and phosphorus, the results for the raw milk being compared with those for the heated milk. For comparative purposes the method would appear to have much to recommend it, though the values obtained by it for raw milk—24·18–30·64 per cent. of the calcium diffusible, and 28·70–38·38 per cent. of the phosphorus diffusible—are probably lower than the absolute values.

The mineral content of milk.

Broadly speaking the mineral content of milk may be estimated by two methods: (1) directly, by analysis of the milk, (2) indirectly, by analysis of milk ash.

From the point of view of analytical technique the second method is the easier, since determinations by the first method involve the difficulty of separating the organic and inorganic constituents. It should be remembered, however, that the first method should, *a priori*, give a truer representation of the conditions actually present in the milk, for by the process of incineration substances of both organic and inorganic origin are included in the ash, though the estimation regards them as inorganic only.

Thus the CO_2 of the carbonates in milk ash is probably derived mostly from organic constituents and the sulphur and phosphorus are undoubtedly of protein origin to a large extent. A certain loss of volatile constituents and the destruction of citric acid also occurs. Porcher and Chevallier(5) discuss the subject at some length and give some experimental results. They point out that the loss due to the volatility of the chlorides may be considerable. Thus, by determining the chloride in milk as sodium chloride and then in the ash obtained after incineration, using the same method each time, they found that milk containing 2·867 g. per litre of NaCl gave only 1·287 g. in the ash. In another experiment small quantities of sodium chloride were added to solutions of calcium caseinate and the mixture desiccated and calcined in the muffle to a white ash. It was found that losses of chloride, amounting to 66·6 per cent. and 71·0 per cent. NaCl, respectively, occurred in duplicate determinations.

Phosphates of the alkalies would be transformed during calcination, monopotassium metaphosphate and dipotassium pyrophosphate, for instance, losing water and being converted into orthophosphates. Opinions differ as regards the extent to which organic phosphorus contributes towards the phosphates of the ash. Thus Porcher and Chevallier (*loc. cit.*) state that 20 per cent. of the phosphate of the ash may be of organic origin, Jordan, Hart and Patten(6) give tables which indicate that the figure is 20–25 per cent., Forbes and Keith's results(7) lead to the value 50 per cent., Lenstrup's results(8) to 19 per cent., while Van Slyke and Bosworth(9) give analyses which indicate as high a value as 77 per cent. This inconsistency in results is no doubt partly due to the fact that the casein content of milk fluctuates considerably but is much more due to the difficulty in separating the organic and inorganic phosphorus of milk and to the different methods employed by different workers for that purpose. However, there is no doubt that calcination of the casein leads to an increase in the inorganic phosphate of the ash, for the organically combined phosphorus of the casein combines with the bases present and appears as phosphate.

The marked destruction due to incineration occurs in the proteins and citrates. In addition to the phosphorus of the casein part of the sulphur of the

casein and albumin appears in the ash in an inorganic form. A part is lost in the form of volatile sulphur compounds, but the remainder is transformed into sulphates.

It should be remembered therefore that, although an analysis of milk ash may give accurate figures for the total quantities present of some of the inorganic constituents of milk, for others the figure is too low or too high. Moreover no information is gained as to the state of combination of the acids and bases in milk, whether they are present in true solution, adsorbed to the surfaces of protein particles, or in colloidal suspension—considerations which are very important in connection with the physico-chemical problems of milk. Other methods have therefore to be devised for their elucidation, and in recent years the problem has been approached from several angles by a number of workers. So far, however, detailed knowledge is distinctly lacking.

The ash of milk.

The ash of milk is obtained as a white powder when the liquid is evaporated and the residue incinerated at a low red heat. It contains most of the mineral constituents of the milk, but, owing to the disruptive effect of the heat, it does not contain all of them nor do those which are left exist in the same condition in the ash as they do in the original milk.

Table XI shows the figures obtained by various workers for the percentage of ash in milk.

Table XI. *Percentage of ash in milk.*

Author	Percentage
Richmond (1)	0·75
Babcock (2)	0·72
Orla-Jensen (3)	0·72–0·77
Deysher (4)	0·70

Table XII gives the percentage composition of the ash of milk as determined by different workers. These figures show that there is an excess of bases over acids, which accounts for the fact that the ash gives an alkaline reaction. They show too that potassium is the inorganic element present in milk in greatest quantity—a fact which is often overlooked because of the overshadowing importance of calcium and phosphorus. The lime and phosphoric acid together amount to almost 50 per cent. of the ash.

Table XII. *Percentage composition of the ash in milk.*

Investigator	K_2O	CaO	Na_2O	MgO	Fe_2O_3	P_2O_5	Cl	SO_3	CO_2
Richmond(28)	28·71	20·27	6·67	2·80	0·40	29·33	14·00	Trace	0·97
Babcock(28)	25·02	20·01	10·01	2·42	0·13	24·29	14·28	3·84	—
Schrodt and Hansen(28)	25·42	21·45	10·94	2·54	0·11	24·11	14·60	4·11	—
Fleischmann(28)	25·71	24·68	11·92	3·12	0·31	21·57	16·38	—	—
Storch(28)	25·31	21·93	9·44	2·87	—	28·69	13·73	—	—
Orla-Jensen(28) (mean)	23·63	27·32	5·82	2·42	—	26·89	13·57	2·96	—
Schepang(29)	30·33	20·90	8·41	2·25	0·05	24·80	14·55	2·55	—

Minor constituents of the ash. In addition to the elements already discussed, which form the major portion of the ash of milk, the following have been

identified by micro-analytical methods as being present in very small quantities: iron, copper, zinc, aluminium, manganese, and iodine. Wright and Papish (10) made an exhaustive spectrographic examination of the ash of milk and found small but definite traces of silicon, boron, titanium, vanadium, rubidium, lithium, and strontium.

Most of these elements which are present in minute quantities are probably unimportant, but three of them—copper, iron, and iodine—demand greater consideration on account of their effects either in nutrition or in the chemistry of milk and its products.

Supplee and Bellis (11) found the copper content of milk from individual cows to vary from 0·20 to 0·80 mg. per litre. In composite samples of milk from several hundred cows the maximum copper content was 0·6 mg. per litre and the minimum 0·4 mg., the average being 0·45 mg. It has been shown by several authors (*e.g.* Quam and Hellwig (12), Quam, Soloman and Hellwig (13), King and Etzel (14), Supplee and Bellis (15), Rice and Miscall (16)) that milk is capable of dissolving appreciable quantities of copper from plant such as pipes, coolers, and pasteurising pans, and that therefore the copper content of milk may be artificially increased during its passage through a plant having exposed copper surfaces. The figures given above are, however, for the natural copper content of milk. This does not appear to be affected by the nature of the feeding.

The iron content of milk also varies considerably with the individual cow. Thus Trunz (17) found in one cow's milk a variation in the iron content from 19 to 45 mg. per litre and in the milk from another cow a variation from 9 to 23 mg. per litre. This author also found in one cow a tendency for the iron to increase at the end of lactation and Nottbohm and Dörr (18) made a similar observation. The quantity of iron in cow's milk is approximately one-fourth of that in human milk, and it appears to be independent of the nature of the feed.

The range of variation of the mineral constituents

The composition of milk is liable to fluctuation through the operation of several different factors, and it is important that one should know the range of variation exhibited by each one of the mineral constituents. Collected data of this nature appear to be missing and they are really indispensable when considering whether observed differences are due to a specific effect being studied at the time or whether they are due to normal variations which may be expected in any case. For example, in feeding experiments designed to discover the influence of administering certain substances on the composition of the milk, it is essential to know to what extent any differences noted are due to "normal" fluctuations, outside the control of the experiment, and to what extent they are due to direct results of feeding.

Variations in the mineral constituents are a great factor in the successful handling of milk in condenseries. Very small alterations in the relative proportions of some of the constituents may lead to serious trouble during the

sterilisation of evaporated milk, due to alteration in the temperature of heat coagulation. Similar considerations no doubt affect other processes of condensing and drying, for it is well known that success attends the operations connected with some batches of milk much more than with others.

In view of these facts data from the literature have been accumulated in order to show the range of variation exhibited by the various mineral constituents.

Calcium. Cranfield, Griffiths and Ling(31) give tables showing the frequency distribution of CaO, compiled from analyses of samples from individual cows in 15 herds. These figures are reproduced in Table XIII. It may be seen that the extremes of variation are 0·135–0·1399 per cent. CaO for one cow and 0·225–0·2299 per cent. CaO for one other cow. On the other hand 316 cows out of a total of 673 are within the range 0·175 to 0·1899 per cent. CaO, which may therefore be taken as a fairly representative average value. Crichton(38) states that for 20 Ayrshire cows 220 samples of milk drawn over three lactation periods showed a range of variation in the CaO content from 0·126 to 0·212 per cent., with an average value of 0·166 per cent. Sommer(58) gives 0·125 and 0·291 per cent. as the lowest and highest values respectively for the CaO content of samples of milk analysed by himself.

Table XV shows the minimum, maximum and mean values obtained by a number of workers for the CaO content of milk.

Phosphorus. In the figures compiled by Cranfield, Griffiths and Ling (*loc. cit.*) it may be seen (Table XIV) that the extremes of variation for total P_2O_5 are 0·175–0·1799 per cent. in the case of one cow and 0·305–0·3099 in the case of one other cow; 540 cows out of a total of 673 are within the range 0·210 to 0·2549. Sommer (*loc. cit.*) gives 0·155 and 0·273 per cent. for the lowest and highest values of the P_2O_5 content of samples of milk analysed by himself.

Crichton (*loc. cit.*) gives a range of variation from 0·166 to 0·291 per cent. in the P_2O_5 content of 220 samples of milk drawn from 20 Ayrshire cows.

Table XVI shows the minimum, maximum and mean values obtained by various workers for total P_2O_5.

Magnesium. Figures for the magnesium content of milk are not so numerous as those for calcium and phosphorus. Sommer (*loc. cit.*) states that the lowest and highest values recorded in his own analyses for MgO content have been 0·005 and 0·039 per cent. respectively. Table XVII gives the minimum, maximum and mean values recorded by various workers for MgO content.

Even from the distinctly meagre data available it is apparent that the magnesium content of milk is subject to very wide variations.

Sodium, potassium and chlorine. Tables XVIII, XIX and XX show the minimum, maximum and mean values obtained by different workers for Na_2O, K_2O and Cl contents respectively. Results recorded for these constituents are extremely few.

Table XXI shows a summary of the highest and lowest values recorded for each of the mineral constituents, so far as a search in the literature has revealed.

Table XIII. *Frequency distribution of lime (Cranfield, Griffiths and Ling).*

Herd	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
CaO %																Total
0·135–0·1399	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1
0·140–0·1449	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0·145–0·1499	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	3
0·150–0·1549	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	2
0·155–0·1599	—	—	2	—	—	1	1	—	—	—	—	—	—	5	—	9
0·160–0·1649	—	—	5	—	1	3	2	—	—	—	—	—	—	11	—	22
0·165–0·1699	—	2	2	4	7	3	3	3	1	—	2	—	—	10	—	37
0·170–0·1749	6	6	16	5	10	10	7	2	2	—	6	—	1	9	1	81
0·175–0·1799	8	6	8	7	8	17	9	4	10	5	3	7	—	13	4	109
0·180–0·1849	7	11	10	11	13	9	5	4	2	3	3	5	3	6	3	95
0·185–0·1899	7	9	8	7	20	18	4	5	5	5	6	4	10	4	—	112
0·190–0·1949	7	9	6	7	12	13	3	3	—	2	2	2	2	4	—	72
0·195–0·1999	2	10	3	6	11	13	6	—	1	2	—	2	1	—	—	57
0·200–0·2049	3	3	—	1	6	5	2	1	1	2	—	2	2	1	—	29
0·205–0·2099	2	3	—	2	4	2	1	2	1	1	2	1	—	—	—	21
0·210–0·2149	—	4	—	—	1	4	1	—	—	1	—	—	—	—	—	11
0·215–0·2199	—	2	—	—	5	—	—	—	—	1	—	—	—	—	—	8
0·220–0·2249	—	—	—	—	—	—	—	1	1	—	—	1	—	—	—	3
0·225–0·2299	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Total	42	66	60	51	98	98	44	25	24	22	24	24	19	68	8	673
Mean percentages	0·185	0·190	0·178	0·183	0·188	0·186	0·182	0·185	0·184	0·192	0·183	0·188	0·188	0·171	0·178	0·184

Table XIV. *Frequency distribution of phosphoric acid (Cranfield, Griffiths and Ling).*

Herd	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
P_2O_5 %																Total
0·175–0·1799	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1
0·180–0·1849	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1
0·185–0·1899	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0·190–0·1949	—	—	—	—	—	1	—	1	—	—	1	—	—	3	—	6
0·195–0·1999	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1
0·200–0·2049	1	—	3	—	—	3	2	1	—	—	—	—	—	4	—	14
0·205–0·2099	4	1	1	6	3	4	2	—	—	—	1	—	—	5	—	27
0·210–0·2149	4	1	1	5	3	11	1	3	1	—	1	2	—	8	1	42
0·215–0·2199	4	1	5	8	7	16	5	2	4	—	3	—	—	11	1	67
0·220–0·2249	12	2	3	5	12	14	2	6	1	—	2	1	—	12	2	74
0·225–0·2299	3	3	7	2	11	17	—	1	4	1	4	3	1	11	2	70
0·230–0·2349	3	1	4	6	13	5	4	3	1	—	3	3	2	4	1	53
0·235–0·2399	3	8	5	5	15	4	5	2	3	4	3	—	4	4	—	65
0·240–0·2449	3	6	9	6	9	8	4	2	3	2	2	5	4	3	—	66
0·245–0·2499	2	7	4	3	9	7	9	—	4	3	—	2	—	2	—	52
0·250–0·2549	2	13	11	3	6	5	1	1	1	3	3	2	—	—	—	51
0·255–0·2599	1	12	2	1	4	1	1	1	1	2	—	1	3	—	—	30
0·260–0·2649	—	7	1	—	3	1	2	1	—	4	—	1	1	—	—	21
0·265–0·2699	—	3	1	1	1	1	1	—	—	1	1	3	1	—	—	14
0·270–0·2749	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	2
0·275–0·2799	—	1	2	—	2	—	3	—	—	—	—	—	—	—	—	8
0·280–0·2849	—	—	1	—	—	—	—	—	—	1	—	1	—	—	—	3
0·285–0·2899	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0·290–0·2949	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1
0·295–0·2999	—	—	—	—	—	—	—	1	—	1	—	—	—	—	—	2
0·300–0·3049	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0·305–0·3099	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1
Total	42	66	60	51	98	98	44	26	23	22	24	24	19	68	7	672
Mean percentages	0·231	0·247	0·238	0·229	0·235	0·227	0·238	0·232	0·234	0·253	0·230	0·243	0·250	0·220	0·222	0·234

Table XV. *Minimum, maximum and mean values obtained by different workers for the* CaO *content of cow's milk.*

Investigators	Remarks	Min.	Max.	Mean
Trunz(17)	Cow No. 1	0·168	0·222	0·191
	Cow No. 2	0·161	0·194	0·179
Katagama(24)	For highly bred races	0·143	0·227	0·167
	For primitive races	0·150	0·204	0·178
Hart, McCollum and Humphrey(42)	1 cow, period of 65 days	0·118	0·136	0·128
Forbes and Beigle(25)	6 cows, same stage of lactation	0·115	0·150	—
	6 cows, different stages of lactation	0·104	0·153	0·128
Forbes, *et al.*(49)	6 cows	0·113	0·179	0·144
Forbes, *et al.*(59)	6 cows	0·144	0·179	0·164
Hess, Unger and Supplee(55)	1 cow (before and after grazing)	0·138	0·165	—
Meigs, *et al.*(27)	Cow No. 1, 11 months' lactation	0·122	0·160	0·140
	Cow No. 2, 5 months' lactation	0·174	0·203	0·185
Sommer and Hart(30)	Cow No. 1, whole lactation	0·164	0·291	0·189
	Cow No. 2, 9 months' lactation	0·157	0·190	0·174
	Cow No. 3, 10 months' lactation	0·157	0·249	0·198
	3 cows	0·161	0·202	0·181
Cranfield, Griffiths and Ling(31)	673 cows	0·135–0·140	0·225–0·230	0·184
Proks(56)	Separate quarters of 5 cows	0·203	0·265	—
Davies and Provan(53)	14 cows	0·152	0·206	0·173
Cranfield and Ling(37)	Abnormal cow, 2 lactations	0·092	0·192	0·143
Sommer(58)	—	0·125	0·291	—
Crichton(38)	20 Ayrshire cows, 3 lactations	0·126	0·212	0·166

Table XVI. *Minimum, maximum and mean values obtained by different workers for the* P_2O_5 *content of cow's milk.*

Investigators	Remarks	Min.	Max.	Mean
Trunz(17)	Cow No. 1	0·190	0·235	—
	Cow No. 2	0·204	0·230	—
Jordan, Hart and Patten(6)	Cow No. 1, 25 days' lactation	0·170	0·215	0·198
	Cow No. 2, 7 days' lactation	0·229	0·238	0·235
Katagama(24)	For highly bred races	0·180	0·273	0·216
	For primitive races	0·153	0·296	0·225
Hart, McCollum and Humphrey(42)	1 cow, period of 65 days	0·164	0·180	0·173
Lauder and Fagan(45)	Several cows	0·19	0·33	—
Forbes and Beigle(25)	6 cows, same stage of lactation	0·156	0·201	—
	6 cows, different stages of lactation	0·146	0·201	0·173
Forbes, *et al.*(49)	6 cows, different stages of lactation	0·147	0·206	0·179
Forbes, *et al.*(59)	6 cows, different stages of lactation	0·195	0·231	0·215
Hess, Unger and Supplee(55)	1 cow (before and after grazing)	0·158	0·190	—
Sheehy(60)	6 cows	0·162	0·236	0·205

Table XVI continued. *Minimum, maximum and mean values obtained by different workers for the* P_2O_5 *content of cow's milk.*

Investigators	Remarks	Min.	Max.	Mean
Lenstrup(61)	15 samples, mixed milk from 30 cows	0·205	0·227	0·219
Meigs, *et al.*(27)	Cow No. 1, 11 months' lactation	0·179	0·224	0·199
	Cow No. 2, 5 months' lactation	0·245	0·282	0·260
Sommer and Hart(30)	Cow No. 1, whole lactation	0·196	0·273	0·217
	Cow No. 2, 9 months' lactation	0·236	0·277	0·258
	Cow No. 3, 10 months' lactation	0·211	0·263	0·234
	3 cows	0·207	0·285	0·236
Zaykowsky(44)	1 cow, 7 weeks' lactation	0·189	0·239	—
Cranfield, Griffiths and Ling(31)	673 cows	0·175–0·180	0·305–0·310	0·234
Davies and Provan(53)	14 cows	0·182	0·303	0·240
Sommer(58)	—	0·155	0·273	—
Crichton(38)	20 Ayrshire cows, 3 lactations	0·166	0·291	0·229
Cranfield and Ling(37)	Abnormal cow, 2 lactations	0·108	0·237	0·178

Table XVII. *Minimum, maximum and mean values obtained by different workers for the* MgO *content of cow's milk.*

Investigators	Remarks	Min.	Max.	Mean
Trunz(17)	Cow No. 1	0·0183	0·0253	—
	Cow No. 2	0·0195	0·0284	—
Hart, McCollum and Humphrey(42)	1 cow, 65 days' lactation	0·013	0·020	0·018
Forbes and Beigle(25)	Same cow, different periods of lactation	0·0133	0·0168	—
	6 cows	0·0133	0·0211	0·0162
Forbes, *et al.*(49)	6 cows	0·0151	0·0212	0·0178
Forbes, *et al.*(59)	6 cows	0·020	0·025	0·022
Hess, Unger and Supplee(55)	1 cow (before and after grazing)	0·005	0·009	—

Table XVIII. *Minimum, maximum and mean values obtained by different workers for the* Na_2O *content of cow's milk.*

Investigators	Remarks	Min.	Max.	Mean
Trunz(17)	Cow No. 1	0·036	0·050	0·043
	Cow No. 2	0·044	0·087	0·057
Forbes and Beigle(25)	6 cows	0·0461	0·0807	0·0609
Hess, Unger and Supplee(55)	1 cow (before and after grazing)	0·051	0·056	—
Sommer(58)	—	0·070	0·090	—

Table XIX. *Minimum, maximum and mean values obtained by different workers for the* K_2O *content of cow's milk.*

Investigators	Remarks	Min.	Max.	Mean
Trunz(17)	Cow No. 1	0·148	0·174	0·164
	Cow No. 2	0·172	0·186	0·180
Forbes and Beigle(25)	6 cows	0·1757	0·2227	0·1925
Hess, Unger and Supplee(55)	1 cow (before and after grazing)	0·150	0·157	—
Sommer(58)	—	0·175	0·190	—

Table XX. *Minimum, maximum and mean values obtained by different workers for the* Cl *content of cow's milk.*

Investigators	Remarks	Min.	Max.	Mean
Trunz(17)	Cow No. 1	0·074	0·100	0·085
	Cow No. 2	0·099	0·171	0·126
Forbes and Beigle(25)	6 cows	0·0726	0·1274	0·1013
Hess, Unger and Supplee(55)	1 cow (before and after grazing)	0·054	0·097	—
Sommer(58)	—	0·100	0·120	—

Table XXI. *Summary of lowest and highest values obtained by different workers for inorganic constituents of milk.*

	Investigators	Lowest %	Highest %
CaO	Cranfield and Ling(37)	0·092	—
	Sommer(58)	—	0·291
MgO	Hess, Unger and Supplee(55), Sommer(58)	0·005	—
	Sommer(58)	—	0·039
K_2O	Trunz(17)	0·148	—
	Forbes and Beigle(25)	—	0·211
Na_2O	Trunz(17)	0·036	—
	Sommer(58)	—	0·090
Cl	Hess, Unger and Supplee(55)	0·054	—
	Trunz(17)	—	0·171
P_2O_5	Cranfield and Ling(37)	0·108	—
	Lauder and Fagan(45)	—	0·33

Range of variation of soluble Ca, Mg *and* P. Tables XXII, XXIII and XXIV show the highest, lowest and mean values for the soluble Ca, Mg and P, expressed as the percentage of the total Ca, Mg and P, respectively, as obtained by various workers.

Table XXII. *Soluble* Ca *as percentage of total* Ca.

Investigators	Min.	Max.	Mean	No. of determinations	Method of determination
Van Slyke and Bosworth(62)	23·13	45·69	32·96	18	Filtrate through Pasteur-Chamberland filter
György(67)	—	—	15·62	1	Dialysed against water for 36 hours
Mattick(73)	24·18	30·64	26·84	11	Dialysed against 3 % saline solution for 18 hours

Table XXIII. *Soluble* Mg *as percentage of total* Mg.

Investigators	Min.	Max.	Mean	No. of determinations	Method of determination
Van Slyke and Bosworth(62)	64·40	91·16	78·88	16	Filtration through Pasteur-Chamberland filter

Table XXIV. *Soluble* P *as percentage of total* P.

Investigators	Min.	Max.	Mean	No. of determinations	Method of determination
Van Slyke and Bosworth(62)	26·98	64·31	44·05	17	Filtration through Pasteur-Chamberland filter
György(67)	39·8	53·1	44·95	4	Filtration through de Hain filter
György(67)	—	—	17·00	1	Dialysed against water for 36 hours
Mattick(73)	28·70	38·38	32·94	11	Dialysed against 3 % saline solution for 18 hours

The number of investigators is small and their respective methods of determination considerably different, so that only tentative conclusions should be drawn from the data, pending a more complete investigation. The values for soluble calcium range from 15·62 to 45·69 per cent., for soluble magnesium from 64·40 to 91·16 per cent. and for soluble phosphorus from 26·98 to 64·31 per cent. The range of variation shown by any one investigator or set of investigators is in nearly all cases very considerable, so that the available evidence points to the conclusion that the proportion of these soluble constituents in milk is a highly variable quantity.

Factors causing variation.

A number of factors are responsible for differences in the composition of milk, some of them being physiological factors inherent in the animal while others are due to external conditions. They may be briefly tabulated as follows:

Factors inherent in the animal:

(1) Breed and individuality.
(2) Period of lactation.
(3) Yield.
(4) Differences in different quarters of the same udder.

External factors:

(1) Feeding.
(2) Season of the year.
(3) Disease.
(4) Time of milking.

Influence of breed and individuality. Data on the subject of breed in its relation to the composition of milk are very few, but Grimmer(22) cites two authorities whose opinions are worth noting. Thus Soxhlet(23) expressed the opinion that the more highly bred races give milk low in calcium, but Katagama's experiments(24) do not support this view. For highly bred races the latter found variations between 0·143 and 0·227 per cent. for CaO and between 0·180 and 0·273 per cent. for P_2O_5, while for primitive races he found values between 0·150 and 0·204 per cent. for CaO and between 0·153 and 0·296 per cent. for P_2O_5. As mean values he gives the following figures:

Highly bred races	0·167 % CaO; 0·216 % P_2O_5.
Primitive races	0·178 % CaO; 0·225 % P_2O_5.

The following values (Katagama's) for the average ratio CaO/P_2O_5 are interesting as showing the variations between the different breeds:

Breed	Ratio CaO/P_2O_5	Breed	Ratio CaO/P_2O_5
Red Silesian	1 : 1·42	Voigtlander	1 : 1·26
Dutch	1 : 1·42	African Cross	1 : 1·26
Scheinfeld	1 : 1·39	Simmenthaler	1 : 1·21
Corean	1 : 1·39	East Prussian Dutch	1 : 1·21
Schwyzer	1 : 1·34	Ceylon	1 : 1·18
African	1 : 1·30	Red-brown East Friesian	1 : 1·15
Wilstermarsch	1 : 1·29	Roumanian Cross	1 : 1·15
Roumanian	1 : 1·28	Buffalo	1 : 1·02
Pied East Friesian	1 : 1·28		

Figures for the differences in composition due to individuality are not easy to compute accurately, for it must be remembered that though differences between cows of the same breed may be observed, it is often impossible to say to what extent these differences are due to individuality and to what extent they are caused by any one of the several other factors, such as stage of lactation, state of health, etc., which are known to influence the composition as well. However, that differences in composition of the milk of individual cows do occur is well recognised. The figures in Table XXVII showing the results obtained by Forbes and Beigle (25) for the analyses of the milk of six cows, of the same breed and all in approximately the same stage of lactation, bring out individual differences quite well. Thus in period 1 the ash content varies from 0·6129 to 0·6776 per cent., the calcium content from 0·0740 to 0·0980 per cent., the magnesium content from 0·0080 to 0·0127 per cent., the chlorine content from 0·0726 to 0·1274 per cent. and the phosphorus content from 0·0637 to 0·0778 per cent. Similar differences are recorded for the other two periods.

Influence of period of lactation. Milk undergoes well-marked changes in composition as the period of lactation advances, but although this is a well-known phenomenon, the number of workers who have actually given detailed data for the analysis of milk at definite periods of lactation is surprisingly small. However, some investigators have given figures for some constituents and others for different constituents, while in some cases the results of analyses of milk have been given and it happens that the period of lactation has been stated by the author responsible, though the object of the experiments was not connected with lactation. Thus by a survey of the literature and correlation of various data it has been possible to arrive at some experimental support for deductions of a general nature.

Trunz (17) gives figures for the composition of the ash from the milk of two cows throughout lactation, first for colostrum and then for three divisions of the remaining period. Table XXV shows these results calculated to the per-

Table XXV. *Percentage of mineral constituents in milk throughout lactation (Trunz).*

	Cow No. 1				Cow No. 2			
	Colostrum	Period 1	Period 2	Period 3	Colostrum	Period 1	Period 2	Period 3
Total ash	0·705	0·598	0·599	0·675	0·684	0·651	0·701	0·771
K_2O	0·174	0·168	0·165	0·148	0·176	0·186	0·186	0·172
Na_2O	0·050	0·036	0·036	0·048	0·051	0·044	0·046	0·087
CaO	0·205	0·168	0·169	0·222	0·181	0·161	0·180	0·194
MgO	0·025	0·019	0·017	0·025	0·028	0·020	0·020	0·025
Cl	0·089	0·076	0·074	0·100	0·101	0·099	0·131	0·171
P_2O_5	0·180	0·147	0·151	0·153	0·168	0·162	0·161	0·158

centage in milk, after deducting the sulphur and phosphorus of protein origin. From these we may make the following observations:

(1) The total ash is higher in colostrum and towards the end of lactation than in the intervening period.

(2) Chloride increases quite markedly towards the end of lactation.

(3) Potassium content falls towards the end of lactation, while sodium follows the opposite rule.

(4) Both calcium and magnesium are higher in colostrum and towards the end of lactation.

(5) Phosphoric acid is higher in colostrum than subsequently.

(6) If the ratio CaO/P_2O_5 is calculated for each period we obtain the following values:

	Colostrum	Period 1	Period 2	Period 3
Cow No. 1	1·139	1·143	1·119	1·451
Cow No. 2	1·077	0·994	1·118	1·228

In the case of both cows the ratio is definitely higher at the end of lactation.

Schrodt and Hansen(26) give figures for the composition of milk ash at different periods of lactation—for colostrum, for the milk 1 day after calving, and 10 days after calving, and for the milk from an advanced period of lactation. Their results are shown in Table XXVI, and from these it appears that

Table XXVI. *Composition of milk ash at different periods of lactation (Schrodt and Hansen).*

	Colostrum for day of calving (%)	Milk 1 day after calving (%)	Milk 10 days after calving (%)	Milk from advanced lactation (%)
K_2O	17·40	18·17	24·12	20·61
Na_2O	10·10	11·94	8·72	16·15
CaO	22·99	26·69	22·69	20·97
MgO	6·88	3·07	2·92	2·75
Fe_2O_3	0·42	0·05	Trace	0·19
SO_3	2·82	3·94	4·10	3·74
P_2O_5	34·30	23·87	30·73	22·18
Cl	6·85	16·01	8·30	17·63

calcium and magnesium are high at the beginning of lactation and potassium rather higher a few days after calving than at a later period—results which confirm the findings of Trunz. Phosphoric acid fluctuates considerably, but inasmuch as this value includes phosphorus of protein origin and the corresponding values of protein are not stated, it is impossible to make any deductions as to variations for the inorganic phosphorus.

Forbes and Beigle(25) in a study of the mineral metabolism of the milch cow give figures for the calcium and phosphorus in milk at different periods of lactation for six cows. The cows were freshened 4 to 6 weeks before the experiments began and there were three experimental periods, each of 19 or 20 days' duration, with intervals of 10 days between each. We may therefore take period 1 as corresponding approximately to a phase of lactation between 4 to 6 weeks and 7 to 9 weeks, period 2 between 8 to 10 weeks and 11 to 13 weeks, and period 3 between 12 to 14 weeks and 15 to 17 weeks after parturition. Samples were taken daily throughout each period and kept in a refrigerator at 0° F., all being mixed for a final composite sample for analysis. Details of analyses are not given, but the phosphorus includes both organic and inorganic

phosphorus. The results of analyses are shown in Table XXVII. It should be borne in mind that the experiment covers only a period of 11 weeks altogether and includes neither the beginning nor the end of lactation, so that large differences in composition are not to be expected. On the other hand, however, each analysis is for a composite sample for the milk of 20 consecutive days and therefore any differences observed for the figures of two different periods may be regarded as free from mere daily fluctuations and may be taken as representing true periodic differences. For five cows the calcium content shows a general increase with advance of lactation, which is particularly marked in the case of cow No. 2. Cow No. 1, however, is an exception to this and shows a

Table XXVII. *Variation in percentage of mineral constituents of milk* (*Forbes and Beigle*).

Period	Cow No.	Ash	Na	K	Ca	Mg	S	Cl	P
1	1	0·6776	0·0470	0·1544	0·0980	0·0127	0·0272	0·0772	0·0765
	2	0·6516	0·0420	0·1754	0·0754	0·0092	0·0234	0·1065	0·0765
	3	0·6253	0·0502	0·1542	0·0740	0·0086	0·0183	0·1274	0·0637
	4	0·6430	0·0487	0·1384	0·0912	0·0091	0·0288	0·0992	0·0778
	5	0·6129	0·0428	0·1450	0·0823	0·0080	0·0218	0·1099	0·0737
	6	0·6136	0·0366	0·1493	0·0832	0·0092	0·0216	0·0838	0·0767
2	1	0·6588	0·0373	0·1548	0·0939	0·0113	0·0253	0·0726	0·0730
	2	0·6605	0·0408	0·1679	0·0834	0·0100	0·0257	0·0984	0·0879
	3	0·6139	0·0342	0·1462	0·0818	0·0084	0·0237	0·1041	0·0842
	4	0·7224	0·0538	0·1400	0·1068	0·0100	0·0244	0·1123	0·0768
	5	0·6809	0·0514	0·1443	0·0921	0·0101	0·0233	0·1121	0·0682
	6	0·6943	0·0399	0·1549	0·0953	0·0107	0·0250	0·0896	0·0753
3	1	0·6504	0·0405	0·1568	0·0925	0·0114	0·0290	0·0797	0·0799
	2	0·7101	0·0479	0·1605	0·1093	0·0099	0·0284	0·1016	0·0801
	3	0·6396	0·0488	0·1410	0·0859	0·0082	0·0242	0·1075	0·0649
	4	0·7100	0·0599	0·1438	0·1074	0·0098	0·0278	0·1257	0·0766
	5	0·6582	0·0520	0·1499	0·0864	0·0094	0·0282	0·1212	0·0667
	6	0·6671	0·0401	0·1515	0·0983	0·0099	0·0285	0·0944	0·0794

decrease. The magnesium is generally lower in the first period than in the other two periods, though cow No. 1 is again an exception. The ash content too is lower in the first period than in the later periods and the same cow forms an exception to this rule. In this respect the table is perhaps more striking in bringing out the effect of individuality of the cow, rather than in pointing the way to generalisations as to change in composition of the milk with period of lactation.

Meigs and co-workers(27) give figures for the calcium, phosphorus and nitrogen of the milk of two cows at different periods of lactation. From their particulars as to the dates when the cows calved and when experiments commenced it is possible to calculate the period of lactation corresponding to each analysis. This has been done and the results are shown in Table XXVIII. Each analysis is for a composite sample covering the period shown. The figures for phosphorus include both organic and inorganic phosphorus, and though the nitrogen is shown this includes both casein and albumin so that unfortunately it is impossible to calculate the figure for inorganic phosphorus separately. In the case of cow No. 54, it may be seen that the calcium is slightly higher in the earlier stage of lactation (41 to 77 days) than in the advanced stage (240–362

days), and again increases at the very end of lactation. In the case of cow No. 7, the calcium is considerably higher all the way through the experiment, but although the figures show appreciable fluctuations no conclusions of a general nature can be drawn. It is interesting to see that in neither case does the phosphorus content follow the nitrogen content.

Sommer and Hart (30), considering the factors responsible for fluctuations in the composition of milk and therefore indirectly responsible for differences in the temperature of heat coagulation, studied the composition of the milk from three cows throughout lactation.

Table XXVIII. *Calcium, phosphorus and nitrogen content of milk of two cows at different periods of lactation (Meigs and co-workers).*

Cow No. 54				Cow No. 7			
Days after calving	Ca	P	N	Days after calving	Ca	P	N
41–45	0·109	0·098	0·495	54–61	0·124	0·112	0·555
45–49	0·111	0·090	0·477	61–68	0·126	0·111	0·551
49–53	0·106	0·092	0·447	68–75	0·128	0·112	0·564
53–57	0·103	0·093	0·467	75–82	0·132	0·115	0·571
57–61	0·105	0·096	0·492	82–89	0·132	0·111	0·585
61–65	0·104	0·092	0·472	89–96	0·134	0·115	0·591
65–69	0·106	0·090	0·477	96–103	0·129	0·110	0·581
69–73	0·104	0·094	0·475	103–110	0·130	0·111	0·604
73–77	0·102	0·092	0·475	110–117	0·130	0·115	0·588
240–247	0·098	0·085	0·537	117–124	0·139	0·117	0·594
247–254	0·098	0·085	0·534	124–131	0·131	0·116	0·625
254–261	0·095	0·083	0·527	131–138	0·131	0·112	0·618
261–268	0·099	0·083	0·530	138–145	0·134	0·114	0·645
268–275	0·099	0·083	0·532	145–152	0·126	0·112	0·636
275–282	0·099	0·083	0·531	152–159	0·129	0·111	0·640
282–299	0·100	0·084	0·532	159–166	0·130	0·113	0·643
299–306	0·096	0·081	0·547	166–173	0·128	0·113	0·636
306–313	0·087	0·083	0·538	173–180	0·135	0·119	0·634
313–320	0·094	0·081	0·531	180–187	0·133	0·116	0·637
320–327	0·093	0·079	0·523	187–194	0·135	0·113	0·655
327–334	0·087	0·078	0·527	194–201	0·132	0·111	0·639
334–341	0·089	0·079	0·539	201–208	0·130	0·108	0·607
341–348	0·093	0·085	0·557	208–215	0·135	0·107	0·656
348–355	0·093	0·084	0·539	215–222	0·145	0·123	0·649
355–362	0·097	0·088	0·566	222–229	0·137	0·118	0·636
362–369	0·104	0·090	0·582				
369–376	0·109	0·094	0·600				
376–383	0·114	0·090	0·641				
383–390	0·101	0·078	0·813				

The results of their analyses for calcium and phosphorus—composite samples for each month—are shown in Table XXIX.

This survey of the available data leads to the conclusion that the calcium and magnesium are higher at the beginning and end of lactation than in the intermediate period, and that the inorganic phosphorus decreases while the ratio CaO/P_2O_5 increases towards the end of lactation.

The data which have been shown, however, demonstrate in the first place that considerable fluctuations occur from day to day in the mineral constituents, and in the second place that before definite conclusions can be made with regard to the majority of the constituents a very considerable amount of detailed and comprehensive investigation is essential.

Influence of yield. Several investigators have shown that an inverse relationship exists between the fat content and the daily yield of milk—*e.g.* Hammond and Hawk (19)—but comparatively little has been done with regard to the variations of other constituents in relation to the volume. Taylor and Husband (20) give some interesting data obtained from the study of a goat. The total volume of the 24 hours' secretion was measured and determinations of total protein, casein, albumin plus globulin, non-protein nitrogen, fat, lactose and ash were made on the mixed daily sample. In the first experiment the animal was fed on a constant diet during a period of advanced lactation, when the volume of milk fell steadily, though there were daily fluctuations. The results obtained are recorded graphically in Fig. 3. Two similar experiments on the same animal were made—one at the beginning of lactation when the yield was increasing rapidly and the other at the end of lactation when the yield was decreasing. These results are shown in Figs. 4 and 5. From these and other observations Taylor and Husband formulated the following general principle:

Table XXIX. *Effect of period of lactation on the composition of milk (Sommer and Hart).*

Cow No. 8, freshened Nov. 11			Cow No. 3, freshened Nov. 25			Cow No. 11, freshened Jan. 23		
Date	CaO (%)	P_2O_5 (%)	Date	CaO (%)	P_2O_5 (%)	Date	CaO (%)	P_2O_5 (%)
Nov. 21–26	0·195	0·250	Dec. 9–16	0·185	0·277	Feb. 1–8	0·221	0·263
Dec. 9–16	0·175	0·205	Feb. 1–8	0·165	0·245	Mar. 3–9	0·187	0·244
Feb. 1–8	0·171	0·204	Mar. 3–9	0·169	0·267	Apr. 7–12	0·195	0·239
Mar. 3–9	0·181	0·217	Apr. 7–12	0·174	0·252	May 1–8	0·192	0·234
Apr. 7–12	0·177	0·211	May 1–8	0·157	0·262	June 16–23	0·187	0·227
May 1–8	0·164	0·196	June 16–23	0·160	0·236	July 11–19	0·183	0·225
June 16–23	0·167	0·205	July 11–19	0·181	0·267	Aug. 4–11	0·186	0·231
July 11–19	0·168	0·215	Aug. 4–11	0·190	0·263	Sept. 11–18	0·195	0·235
Aug. 4–11	0·174	0·205	Sept. 11–18	0·187	0·257	Oct. 7–13	0·223	0·247
Sept. 11–18	0·175	0·207				Nov. 3–10	0·157	0·211
Oct. 7–13	0·207	0·197				Dec. 2–9	0·249	0·223
Nov. 3–10	0·218	0·233						
Dec. 2–9	0·291	0·273						

On a diet of constant composition the percentages of all the constituents of the milk, with the exception of the lactose, tend to vary inversely as the daily volume of the milk secreted; the percentage of lactose, while normally very constant, tends to vary directly as the volume, this being particularly apparent at the beginning and end of lactation.

This principle refers only to a general tendency and not, of course, to an absolute rule. Moreover the evidence on which it is based is not extensive enough for it to be regarded as proven. It should be noted, too, that the investigations of Proks (56), Benton (57), and Mattick and Hallett (21) indicate that the four quarters of the udder are separate physiological units (see p. 32), and we should therefore expect any law correlating yield with percentage of constituents to be followed more accurately by each separate quarter than by the udder as a whole, since results obtained from the latter are merely a mean of four distinct units. Mattick and Hallett (*loc. cit.*) give figures for the yield and the percentage composition of the milk from four quarters of a cow over an

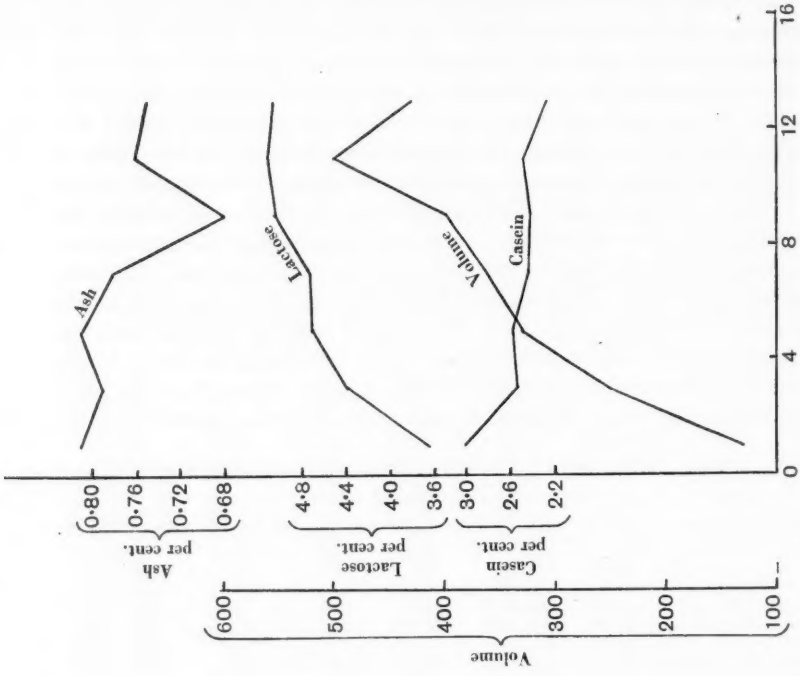


Fig. 4. Effect of variation in volume on percentage composition of milk—Beginning of lactation (Taylor and Husband).

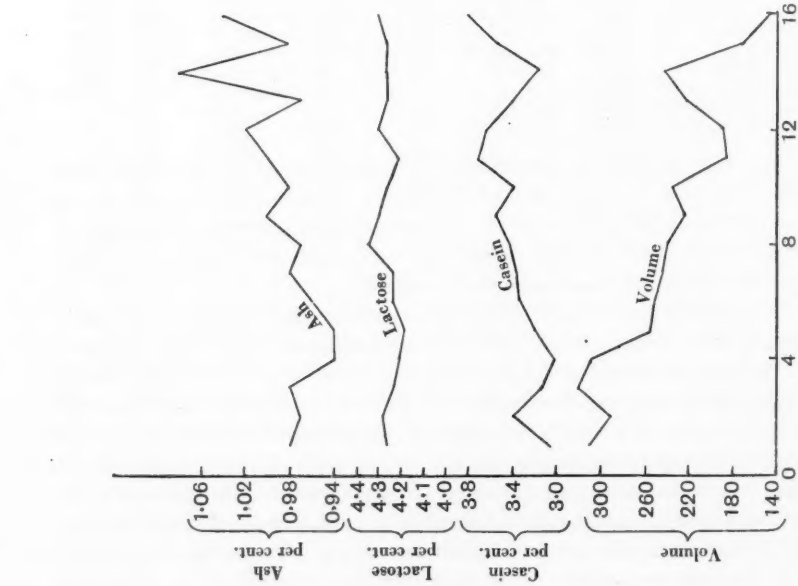


Fig. 3. Effect of variation in volume on percentage composition of milk, with an animal on a diet of constant composition (Taylor and Husband).

entire lactation period. The results for two of the quarters are shown in the form of curves (Figs. 6 and 7) in which yield, casein content and ash content, respectively, are plotted against the sample number. The samples represent alternate morning and evening milkings. Unfortunately, determinations of lactose were not made by these authors, but the curves certainly support the general principle formulated by Taylor and Husband, so far as casein and ash contents are concerned.

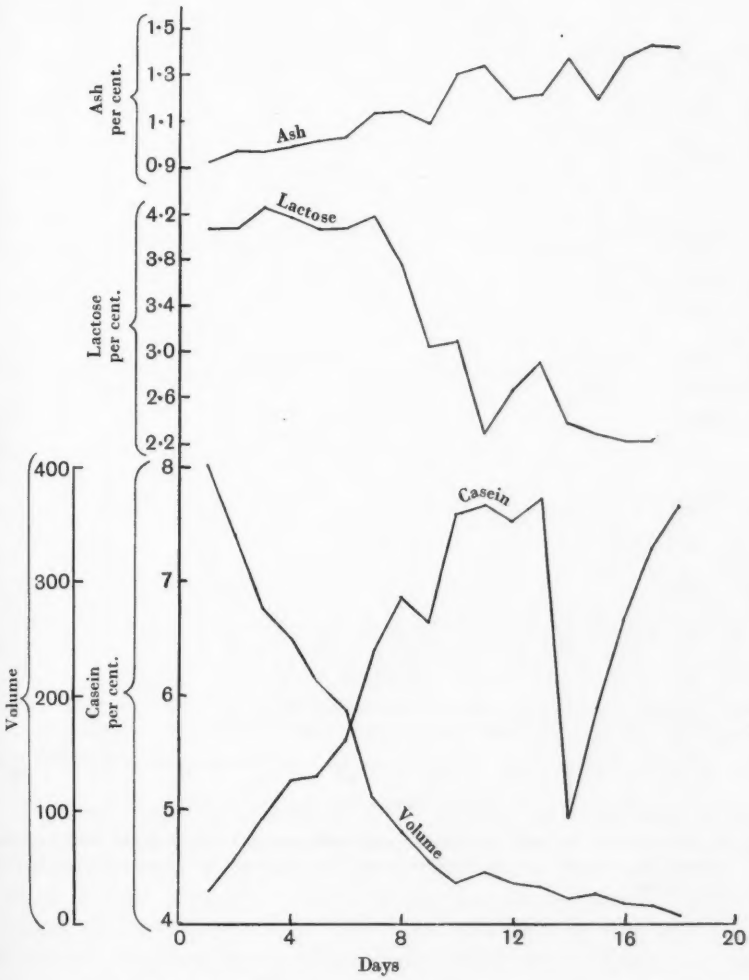


Fig. 5. Effect of variation in volume on percentage composition of milk—End of lactation (Taylor and Husband).

Beginning of lactation (Taylor and Husband).
with an animal on a diet of constant composition (Taylor and Husband).

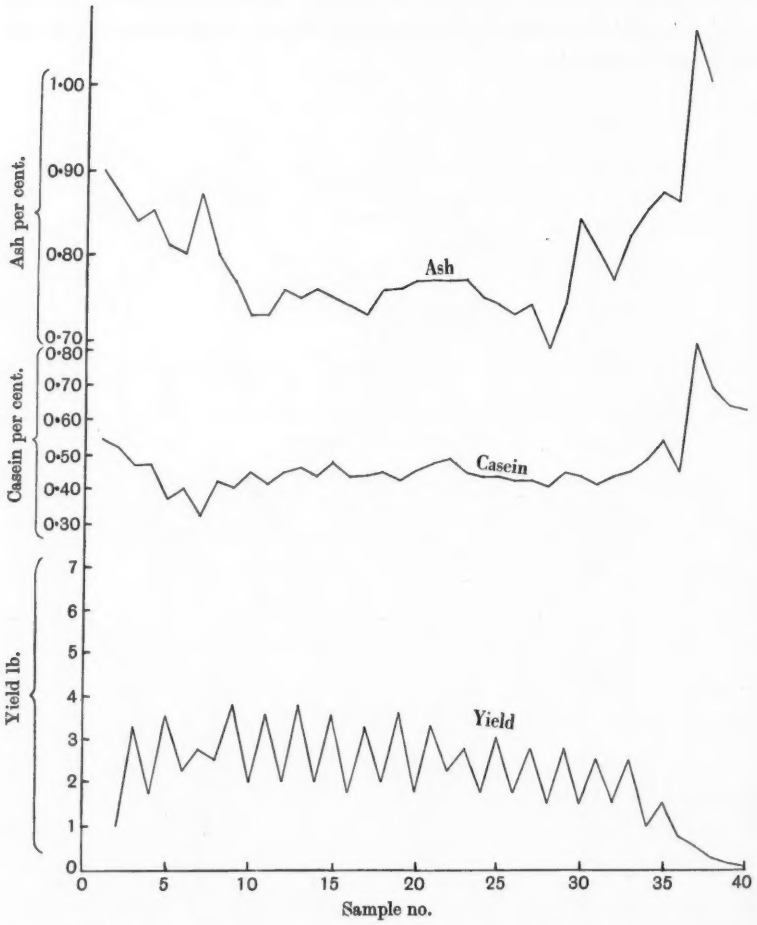


Fig. 6. Relation between yield and the ash and casein contents of milk taken from front left quarter of a cow over an entire lactation period.—(Constructed from figures given by Mattick and Hallett.)

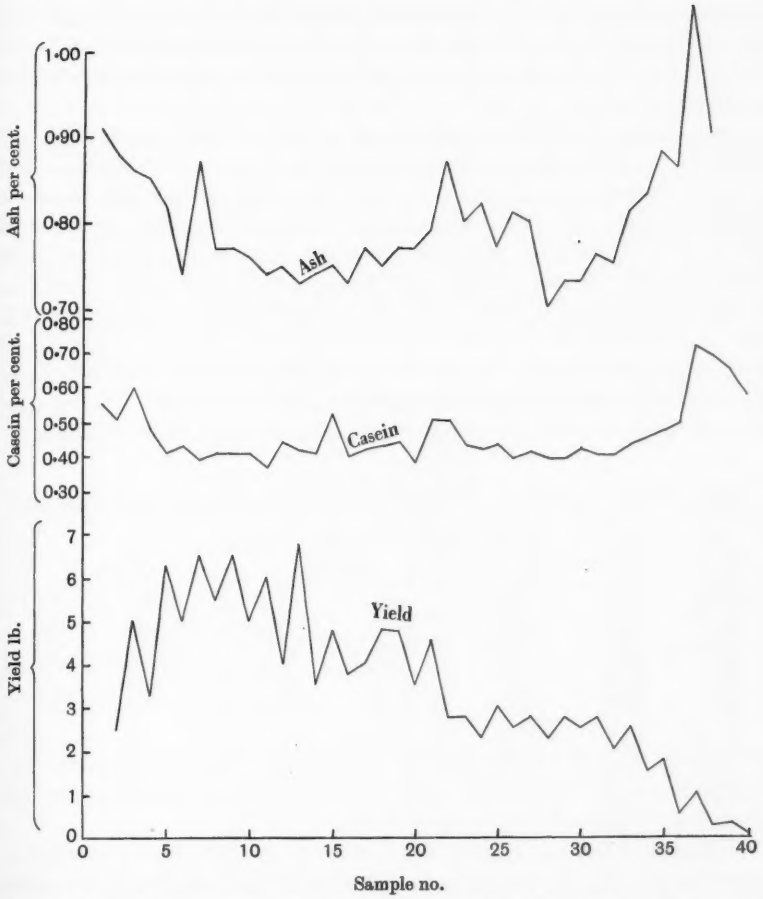


Fig. 7. Relation between yield and the ash and casein contents of milk taken from hind right quarter of a cow over an entire lactation period.—(Constructed from figures given by Mattick and Hallett.)

Differences in different quarters of the udder. The differences in the composition of the milk from different quarters of the same udder are not in themselves likely to be the cause of differences in the composition of samples of milk in general, since of course the milking process normally exhausts all four quarters alike, but it is interesting to note that quite measurable variations occur. Thus Table XXX shows figures for the maximum, minimum and difference in the calcium content of the milk from four quarters in each case of five cows, as obtained by Proks (56). Benton (57), discussing the bearing of such variations on the subject of coagulation, gives

values for the volume of milk, percentage of fat and serum solids, *p*H, alcohol test, etc., of milk from different quarters of the same udder on the same day and on different days. The author concludes from these and similar figures obtained for a number of cows of the herd that the four quarters are separate physiological units.

Mattick and Hallett(21) carried out an extensive investigation of the properties of the milk from the individual quarters of one cow at intervals over an entire lactation period. These authors investigated the yield, titratable acidity, hydrogen-ion concentration, time of coagulation with rennet, total nitrogen, casein nitrogen, amount of butterfat, total solids and ash content. Distinct variations were found, particularly with regard to the yield, the acidity, the time of coagulation with rennet and the percentage of butterfat. Generally speaking the properties of the milk from the two front quarters and from the two hind quarters appeared to be similar, though distinct differences were observed between the milk from the fore and hind quarters. On occasions the milk from one quarter exhibited a striking difference from that from the

Table XXX. *Differences in percentage of calcium in milk from different quarters of the same cow (Proks).*

	Maximum		Minimum		Difference	
Cow No.	Milk	Ash	Milk	Ash	Milk	Ash
169	0·187	25·57	0·185	24·84	0·002	0·73
720	0·189	24·88	0·176	22·06	0·013	2·82
398	0·173	24·46	0·161	23·10	0·012	1·36
326	0·159	24·70	0·153	22·87	0·006	1·83
325	0·153	21·82	0·145	20·72	0·008	1·10

other three, though no physiological explanation could be advanced. This appears to support the contention of Proks and of Benton (*loc. cit.*) that each quarter of the udder functions individually.

Influence of feeding. A great many experiments have been performed to determine whether the composition of milk alters with change in the nature of the food. In general it may be said that the evidence available renders it very doubtful whether the mineral composition of milk is influenced to a measurable extent by changes in the feeding, though two or three investigators claim to have obtained positive results. It is, and probably always will be, a difficult question to decide definitely for several reasons. Apart from the fact that other factors are likely to influence the composition of the milk at the time of feeding, the normal day to day fluctuations tend to mask a small general effect. In addition, supposing that any change does occur as the result of feeding a particular foodstuff, there is probably a lag phase during which no effect is noticeable, and unless the experiment is continued long enough to allow for this an apparently negative result is obtained in any case. For these reasons feeding experiments made to determine the possible effect of change of food or addition of a particular article to the diet should be performed under

the most carefully controlled conditions with a number of cows and over a long enough period to ensure that the differences observed are not due merely to temporary fluctuations of a different nature. These conditions, unfortunately, have been fulfilled but rarely by investigators up to date.

Crichton (38) reviews the question in some detail and lists several workers who have found a negative result. Thus Weiske (39) reported no effect as the result of feeding experiments and von Wendt (40) fed various salts to cows with no result. Neumann (41) fed 100 g. Ca_3PO_4 per head per day and found first a slight fall in calcium and phosphorus content and afterwards a slight increase, which caused him to suggest that previous failure on the part of other experimenters was due to the shortness of the trial carried out. He found an effect only after 3 or 4 weeks. Hart, McCollum and Humphrey (42), from ob-

Table XXXI. *Effect of feeding calcium carbonate and calcium phosphate on* CaO *and* P_2O_5 *content of milk* (*Zaykowsky*).

Cow No.	Period	Extra mineral intake per head per 10 days: CaO g.	Extra mineral intake per head per 10 days: P_2O_5 g.	Milk yield 10 day period (kg.)	Ash (%)	CaO (%)	P_2O_5 (%)
1	1	—	—	113·4	0·711	0·148	0·189
	2	242·7	307·7	121·8	0·784	0·168	0·239
	3	—	—	107·6	0·751	0·149	0·208
	4	288·4	—	119·3	0·767	0·177	0·222
2	1	—	—	100·6	0·797	0·155	0·166
	2	288·4	—	106·0	0·805	0·177	0·184
	3	—	—	99·8	0·800	0·161	0·179
	4	242·7	307·7	103·5	0·822	0·173	0·215
3	1	—	—	107·6	0·747	0·145	0·165
	2	288·4	—	112·9	0·806	0·171	0·175
	3	—	—	110·5	0·768	0·165	0·170
	4	242·7	307·7	127·5	0·837	0·172	0·179
4	1	—	—	111·7	0·797	0·172	0·179
	2	242·7	307·7	120·3	0·876	0·190	0·199
	3	—	—	110·9	0·806	0·175	0·176
	4	288·4	—	118·3	0·920	0·180	0·188

servations on one cow for $3\frac{1}{2}$ months, found that wide variations in potassium, magnesium and phosphorus in the feeding did not influence the percentage of these constituents in the milk. Becker, Eckles and Palmer (43) observed that even under conditions so severe as to cause osteomalacia in the animals the calcium and phosphorus of the milk remained normal in amount and proportion. Crichton, however, does quote one worker who claimed a very definite positive result. Zaykowsky (44), working with four cows, superimposed on the basal ration for 10 day periods either $CaCO_3$ or $CaHPO_4$. Table XXXI gives the results obtained. It should be noted that the increase in CaO and P_2O_5 is accompanied by an increased milk yield and is therefore contrary to the rule formulated by Taylor and Husband (*loc. cit.*).

Lauder and Fagan (45) studied the effect of feeding calcium phosphate to cows and concluded that there was no measurable increase in calcium and phosphorus in the milk. The average value for the P_2O_5 was 0·25 per cent. and

the content of the morning and afternoon milk varied as much as from 0·23 to 0·29 per cent. for the same cow, while over a period of 3 or 4 months a variation from 0·21 to 0·29 per cent. is recorded for the same cow. The lowest value given for any cow is 0·19 per cent. and the highest 0·33 per cent. These fluctuations are sufficient to show the difficulty of determining a small increase due to feeding. Table XXXII shows their results in detail.

Beach and Turner(46) showed that acids in the silage caused no increase in the acidity of the milk, Sommer and Hart(47) included in the diet as much as 120 c.c. of sulphuric acid per day per cow and detected no alteration in the

Table XXXII. *Effect of feeding calcium phosphate on the* P_2O_5 *content of milk (Lauder and Fagan). Results expressed as percentage* P_2O_5.

	Lot 1 (no phosphate).						
	Cow No. 1		Cow No. 2		Cow No. 3		
Date	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	Notes
Dec. 10	0·26	0·24	0·25	0·23	0·24	0·24	Preliminary period
17	0·28	0·24	0·24	0·23	0·21	0·24	
Jan. 7	0·25	0·26	0·23	0·23	0·21	0·25	
14	0·27	0·27	0·24	0·24	0·23	0·22	
Jan. 21	0·27	0·27	0·24	0·24	0·25	0·27	Middle period
28	0·29	0·29	0·26	0·26	0·27	0·27	
Feb. 4	0·28	0·28	0·23	0·29	0·26	0·26	
11	0·30	0·29	0·26	0·21	0·28	0·20	
18	0·29	0·29	0·25	0·24	0·26	0·26	
Feb. 25	0·29	0·29	0·24	0·25	0·25	0·25	End period
Mar. 4	0·29	0·29	0·23	0·24	0·27	0·26	
	Lot 2 (with phosphate).						
	Cow No. 4		Cow No. 5		Cow No. 6		
Date	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	Notes
Dec. 10	0·22	0·19	0·33	0·28	0·25	—	Preliminary period
17	0·20	0·20	0·28	0·27	0·23	0·21	
Jan. 7	0·19	0·19	0·25	0·26	0·22	0·22	
14	0·22	0·21	0·26	0·29	0·22	0·26	
Jan. 21	0·22	0·22	0·28	0·27	0·22	0·22	With additional
28	0·22	0·23	0·29	0·26	0·22	0·22	phosphate
Feb. 4	0·20	0·22	0·27	0·26	0·24	0·24	
11	0·21	0·19	0·29	0·30	0·24	0·23	
18	0·21	0·21	0·28	0·29	0·22	0·22	
Feb. 25	0·22	0·21	0·28	0·28	0·23	0·22	End period, no
Mar. 4	0·20	0·23	0·27	0·29	0·23	0·22	phosphate

acidity of the milk, and Duncombe(48) included 50 c.c. of 85 per cent. phosphoric acid per day per cow with no effect. These results would appear to indicate quite definitely that the acidity of milk is not appreciably altered even by drastic increases in the acidity of the foodstuffs. Duncombe (*loc. cit.*) also obtained negative results on feeding large quantities of lactic, acetic, and butyric acids.

Forbes and co-workers(49) carried out experiments to find the effect of feeding calcium carbonate and ground bone-meal to cows and found no measurable difference in the calcium content of the milk.

Sommer and Hart(30), in a study of the factors affecting the heat coagulation of milk, fed doses of 100 and 200 g. of calcium carbonate per day per head

and found no effect on the titratable acidity, *p*H, alcohol coagulation, rennet coagulation or heat coagulation at 136° C. of the milk. The same experiments were repeated with the addition of cod-liver oil to supply vitamin D to facilitate calcium absorption, but still no effect was observed on the properties or composition of the milk.

In another experiment by the same authors (*loc. cit.*) an attempt was made to determine the effect of feeding grass and grass plus bone-meal to cows on the composition of the milk, three cows being used for the trial. There was first a period of 6 days during which the cows were fed a control ration with hay as roughage. In the second period green grass was substituted for hay to have the same amount of dry matter, the period lasting 17 days. In the third period, lasting 28 days, the cows received bone-meal in addition to the grass. The

Table XXXIII. *Effect of green feed and bone-meal on the composition and properties of milk* (*Sommer and Hart*).

Period	Date	Acidity (%)	Alcohol test	Rennet coag. (min.)	CaO	P_2O_5	Citric acid
			Cow No. 5.				
Control	May 10	0·15	Negative	8½	0·168	0·207	0·107
Grass	May 17	0·16	Negative	11	0·170	0·215	0·137
	23	0·17	Negative	9			
Grass and bone-meal	June 1	0·16	Negative	9	0·161	0·207	0·147
	27	0·16	Positive	6			
			Cow No. 6.				
Control	May 10	0·20	Negative	6	0·200	0·271	0·081
Grass	May 17	0·21	Negative	7	0·202	0·285	0·112
	23	0·21	Negative	6½			
Grass and bone-meal	June 1	0·21	Negative	6	0·197	0·279	0·112
	27	0·22	Positive	5			
			Cow No. 7.				
Control	May 10	0·18	Positive	5	0·187	0·214	0·093
Grass	May 17	0·18	Positive	6	0·172	0·225	0·112
	23	0·19	Positive	6½			
Grass and bone-meal	June 1	0·18	Positive	5½	0·173	0·217	0·150
	27	0·20	Positive	3½			

results are shown in Table XXXIII. Samples for analyses were composite over a period of 6 days at the end of each period. It is to be seen that no increase in the calcium content followed the feeding of the bone-meal. However, although the authors make no reference to the fact there appears to be in the case of each cow a slight but definite increase in phosphoric acid following the feeding of grass. The effect on the alcohol test, rennet coagulation and the citric acid content will be discussed later.

Mattick and Wright (50) studied the effect of oral ingestion of large quantities of calcium chloride, ammonium chloride, sodium bicarbonate and disodium hydrogen phosphate on the yield and composition of cow's milk. They concluded that it was impossible by these means to alter the composition of the milk. With ammonium chloride, however, the milk yield was almost entirely

suppressed and there was a temporary increase in the concentration of the constituents of the milk. The authors attributed these effects to a complete disorganisation of the normal metabolism and the dehydrating influence of the salt.

Taylor and Husband (20) fed (*a*) a high fat diet, (*b*) a high protein diet, (*c*) a high carbohydrate diet, respectively, to a goat in order to determine the effect on the daily volume and composition of the milk. They concluded that diet has no direct influence on the composition of the milk, except in the case of the non-protein nitrogen, which is not a product of the mammary gland. Inasmuch, however, as the diets stimulated the production of milk to different extents (the high protein diet being responsible for the largest yield), and the inverse relationship for protein, fat and ash, and the direct relationship for lactose with respect to volume was observed as postulated previously by these authors (*loc. cit.*), the diet was responsible for an indirect influence.

The effect of feeding cod-liver oil to cows on the calcium and phosphorus content of the milk has been studied by Mattick (51) and by Sheehy and Senior (52). Their results are contradictory. In the experiment by Mattick four cows were fed varying quantities of cod-liver oil (2 to 6 oz. daily) and arachis oil and the milk analysed. The results showed that in the milk of the cows receiving cod-liver oil the percentage of calcium was distinctly higher and that the rennet test required considerably longer time. The evidence cannot be regarded as conclusive owing to the lack of control experiments on the cows before being fed the oil. Moreover such differences in calcium as do appear are within the range which may be normally encountered for milk samples. The author herself points out that insufficient results were obtained to justify definite conclusions.

Sheehy and Senior experimented with three cows during six successive periods of 10 to 13 days each. During the first period the cows received a normal diet, during the second and third periods olive oil in addition, during the fourth period cod-liver oil in addition, during the fifth period olive oil again and during the sixth period a normal diet only. Duplicate analyses of calcium and phosphorus were made on daily samples of the milk. Daily fluctuations were observed but no measurable increase in either constituent due to the feeding of the cod-liver oil was obtained.

Davies and Provan (53) give details of experiments in which they found differences in the composition of milk from cows on pasture and on winter feed respectively. The cows were divided into two groups, group A being fed on a control ration and group B on a low protein ration, both groups being fed thus before being turned out to grass. For analysis the milk of each animal was sampled immediately before grazing commenced and then a fortnight after. Table XXXIV shows the results obtained for the lime, total phosphate and inorganic phosphate content of the milk before and after grazing on lush grass. Total phosphate was determined in the milk after digestion with a mixture of sulphuric and nitric acids, while for estimation of the inorganic phosphate

the proteins were first removed with trichloracetic acid and a colorimetric determination was made on the filtrate.

It will be seen from the table that an increase in lime and inorganic phosphate occurred in the case of twelve animals out of the fourteen, while an increase in total phosphate occurred in the case of thirteen animals after the change over to grass feeding. This is quite a marked result and considering the great lack of positive evidence where feeding experiments are concerned this particular case is outstanding. For this reason it is natural to enquire rather closely into the details of the analyses, etc., and it is disappointing to find in the first place that all results are apparently based on one analysis in each case (it is true it was a composite sample) and in the second place that there is no

Table XXXIV. *Calcium and phosphorus content of milk before and after grazing (Davies and Provan).*

	Lime (CaO) (%)		Total phosphate (P_2O_5) (%)		Inorganic phosphate (P_2O_5) (%)	
	Before grazing	After grazing	Before grazing	After grazing	Before grazing	After grazing
Group A.	EXPERIMENT 1. COWS LET OUT TO GRASS ON 10. V. 27.					
Polly Ddu	0·1614	0·1619	0·2154	0·2144	0·1378	0·1378
Seren 2	0·1621	0·1695	0·2522	0·2752	0·1627	0·1685
Berriers 3	0·1724	0·1771	0·2669	0·2847	0·1773	0·1860
Group B.						
Penwern Ann	0·1822	0·2060	0·2284	0·2578	0·1411	0·1547
Laura 2	0·1988	0·2014	0·2870	0·3030	0·1903	0·1998
Sally Lunn	0·1852	0·1945	0·2793	0·2869	0·1800	0·1881
Group A.	EXPERIMENT 2. COWS LET OUT TO GRASS ON 13. V. 27.					
Rosie	0·1745	0·1824	0·2508	0·2581	0·1684	0·1671
Jean	0·1371	0·1520	0·2176	0·2214	0·1406	0·1504
Group B.						
Berriers 2	0·1800	0·1915	0·2200	0·2412	0·1472	0·1655
Nancy 2	0·1690	0·1740	0·2240	0·2344	0·1515	0·1482
Group A.	EXPERIMENT 3. COWS LET OUT TO GRASS ON 17. V. 27.					
Nancy 1	0·1669	0·1642	0·1824	0·1924	0·1228	0·1290
Peg	0·1427	0·1474	0·1986	0·2032	0·1206	0·1227
Group B.						
Nancy 3	0·1703	0·1824	0·2170	0·2312	0·1340	0·1420
Mabel	0·1634	0·1718	0·2270	0·2410	0·1495	0·1560

evidence as to the consistency of duplicates so as to test the accuracy of the methods employed. However, the fact that fourteen cows were used in the experiment and that appreciable differences are recorded in the case of at least twelve is perhaps fairly strong evidence. The authors point out that the rise in concentration is the more remarkable since it was accompanied by an increased milk yield, and they suggest that it may be due to (1) an improvement in the health of the animals as a result of being left out overnight, or (2) the much higher nutritive value of summer grass compared with winter rations, or (3) a specific effect of some constituents of the grass on the milk glands.

The relation between the nature of the winter ration and the magnitude of the changes occurring when grazing commences are shown in Table XXXV. It will be seen from this that in all three experiments the increase in total and

inorganic phosphate is greater for those animals which were fed on a low protein ration previous to grazing. As regards experiments 1 and 3 this applies to the increase in lime also but in experiment 2 this is not the case. The authors conclude from this that generally speaking the magnitude of the differences observed in the composition of milk on changing over to pasture feeding depend on the nature of the winter feed.

With regard to the possibility that summer grass may have a higher nutritive value than winter rations Orr(54) has investigated the relation of the chemical composition of pasture to its feeding value and gives curves showing the variation in percentage composition with the months of the year. These are reproduced in Fig. 8, and show that there is an increase in the ash content from June to September, a rise in CaO from May to early September followed by a rapid fall, while the P_2O_5 remains fairly constant. The protein content is also considerably higher between June and September. The nutritive value would therefore appear to be higher in the summer months than at other times of the

Table XXXV. *Percentage increase in calcium and phosphorus after grazing* (*Davies and Provan*).

		Increase in CaO (%)			Increase in P_2O_5 (%)			Increase in inorganic P_2O_5 (%)		
Expt.	Group	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
1	A	Nil	0·007	0·004	Nil	0·023	0·014	Nil	0·009	0·005
	B	0·003	0·024	0·012	0·008	0·029	0·027	0·008	0·014	0·011
2	A	0·008	0·015	0·011	0·004	0·007	0·006	Nil	0·010	0·004
	B	0·005	0·012	0·008	0·010	0·021	0·016	Nil	0·018	0·007
3	A	0·003	0·005	0·004	0·005	0·010	0·007	0·002	0·006	0·004
	B	0·008	0·012	0·010	0·014	0·014	0·014	0·007	0·008	0·008

year, but whether this factor would be responsible for an increase in the calcium and phosphorus content of the milk as found by Davies and Provan is at present a matter for speculation.

Hess, Unger and Supplee(55) reported similar results for the beneficial effect of pasture feeding on the calcium and phosphorus content of milk, though in this case the fact that previous to grazing the cows had been fed on a diet devoid of antiscorbutic vitamin adds another factor to those to be considered. Five Holstein cows, freshened about 2 months previously, were used for this experiment and they were fed throughout the winter on a normal ration consisting of ensilage, hay, and concentrates. Beginning on April 30 and for 21 days after the cows were fed on a diet which was ample in all respects except that it was deficient in the antiscorbutic vitamin. The cows were then placed on pasture for 3 weeks, a small quantity of concentrate being given for the first few days only. For analysis a composite sample from a whole day's milking was collected at the end of each 3 week period and in view of the fact that results are based on one such analysis in each case it would obviously be unwise to place too much reliance on any deductions from them. However, the figures for analysis of the milk at the end of each feeding period are given in Table XXXVI and they certainly show a decided increase in the calcium and phosphorus in the milk after pasture feeding.

Table XXXVI. *Composition of milk as affected by fodder (Hess, Unger and Supplee).*

	Antiscorbutic-free fodder (stall feeding)	Pasture
Water	88·38	88·19
Total solids	11·62	11·81
Fat	3·37	3·44
Total protein (N × 6·38)	2·82	2·93
Casein (N × 6·38)	2·28	2·32
Albumin (N × 6·38)	0·42	0·49
Extractive N	0·02	0·02
Lecithin	0·069	0·7
Lactose	4·73	4·56
Citric acid	0·08	0·13
Ash	0·606	0·670
K_2O	0·150	0·157
Na_2O	0·051	0·056
CaO	0·138	0·165
MgO	0·009	0·005
FeO	0·0001	0·0002
P_2O_5	0·158	0·190
SO_3	0·023	0·014
Cl	0·054	0·097

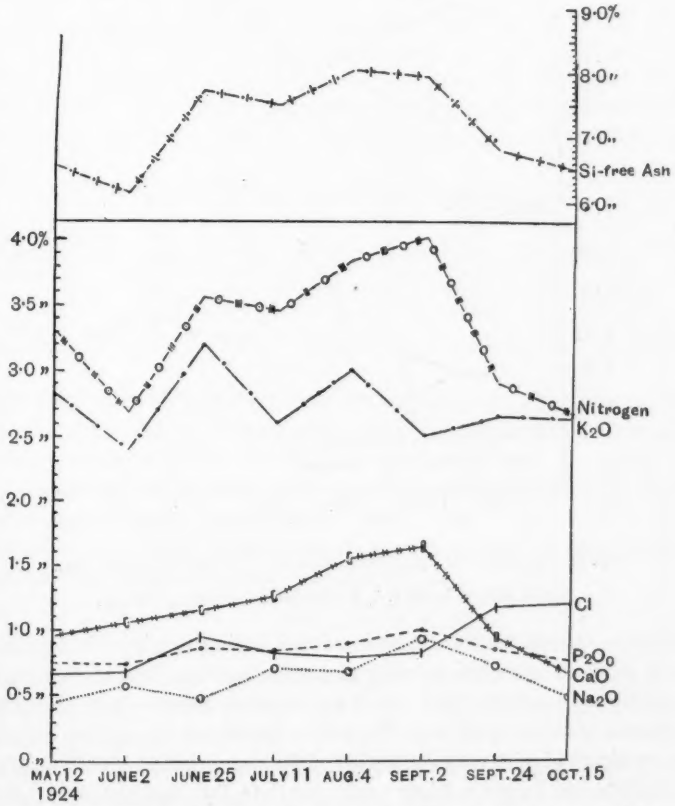


Fig. 8. Variation in the composition of pasture with months of the year (Orr).

This review of the available data indicates that, as far as the effect of feeding specific substances such as calcium carbonate, bone-meal, phosphates or acids is concerned, the composition of milk remains unaltered (except for Zaykowsky's results, which were positive). Concerning the relation of grass feeding and winter feeding to the composition of the milk, however, the few experiments that have been performed in this connection suggest that the calcium and

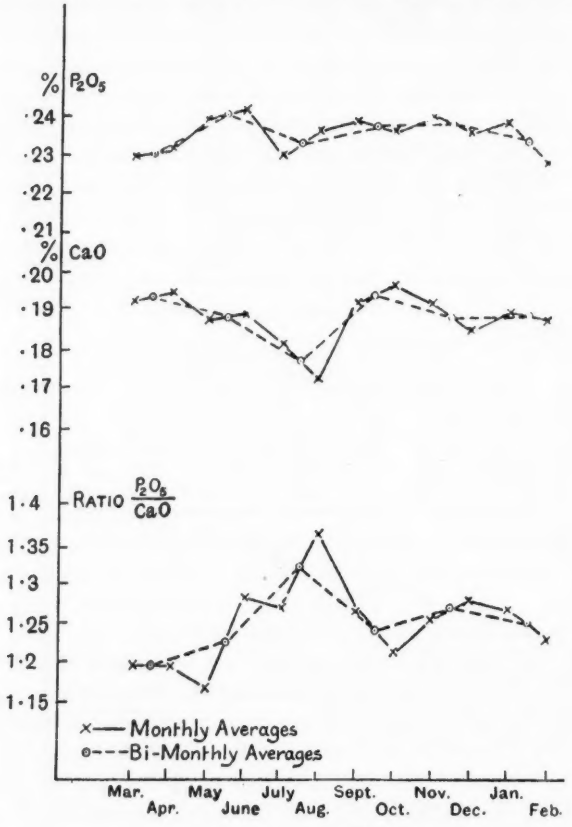


Fig. 9. Correlation of P_2O_5, CaO and ratio $\frac{P_2O_5}{CaO}$ with months of year.
Mixed milk of herds 1, 5, 6 (Cranfield, Griffiths and Ling).

phosphorus content may both be increased during grazing. Before we can arrive at a definite conclusion many more experimental data are essential and they must be of a nature which leaves less room for doubt as to their accuracy.

Influence of season of the year. The only comprehensive analyses correlating the composition of milk with the period of the year appear to be those of Cranfield, Griffiths and Ling (31). Their results for CaO, P_2O_5 and the ratio CaO/P_2O_5 are given in the form of graphs which are reproduced here (Fig. 9). Deter-

minations were made on the milk (not on the ash), and the P_2O_5 includes both organic and inorganic phosphorus. According to these results the P_2O_5 content is influenced but little by the season of the year though there is a slight rise in May and June and a fall in July. Lime on the other hand shows a steady fall in late spring and summer, reaching a low percentage in August, following which a sharp rise occurs. The ratio CaO/P_2O_5 shows a definite variation with the months of the year, reaching its highest value in August. The curve for this ratio is practically the reverse of that for CaO. It is to be noted that the stage of lactation of the cows is not given, so how much of the variation is due to this factor it is impossible to say.

The decrease in calcium takes place at the season of the year when the cows are on pasture. Now in the experiments made by Davies and Provan (p. 36) to determine the effect of feeding pasture on the composition of milk there was strong positive evidence that the calcium content of the milk increased. The results of Davies and Provan and of Cranfield, Griffiths and Ling are therefore contradictory.

Influence of disease. Little information is available as to the effect of disease on the composition and properties of milk but such as there is appears to indicate that the milk may suffer radical changes during the course of illness.

Grimmer (*loc. cit.*) quotes two authors who have given figures for the composition of the ash of so-called "salt milk." Thus Table XXXVII shows the

Table XXXVII. *Analysis of "salt milk."*

Bögold and Stein	K_2O	Na_2O	CaO	MgO	P_2O_5	SO_3	Cl	Fe_2O_3
Sound milk	20·59	13·02	21·55	2·72	26·42	3·66	15·58	—
Salt milk 1	21·69	14·97	20·93	2·21	22·02	3·48	18·65	—
,, 2	10·96	33·17	11·70	2·16	15·63	6·73	25·23	—
,, 3	11·09	31·29	14·61	1·16	15·34	3·92	29·19	—
Hashimoto								
Salt milk	8·94	36·54	7·44	1·74	17·38	1·34	33·63	0·21

results obtained by Bögold and Stein(32) and Hashimoto(33), the values for sound milk (as given by Bögold and Stein) being inserted for comparison. Speaking generally there appears to be a marked decrease in calcium, phosphorus and potassium, an appreciable decrease in magnesium, and a considerable increase in sodium and chlorine.

Schrodt(34) observed the following values for the ash of milk which was drawn from the gradually drying quarter of a cow:

K_2O	Na_2O	CaO	MgO	Fe_2O_3	SO_3	P_2O_5	Cl
8·52	45·85	8·04	1·82	0·97	5·68	9·70	24·35

These figures show the same tendency as those for the analysis of "salt milk"—*i.e.* an increase in sodium and chlorine and a decrease in calcium, phosphorus and potassium.

For a cow with udder catarrh Schrodt (*loc. cit.*) gives the following values:

K_2O	Na_2O	CaO	MgO	SO_3	P_2O_5	Cl
10·56	24·92	16·17	2·70	1·56	24·56	24·52

Storch (35) investigated the composition of the milk from tuberculous cows and found that it exhibited considerable deviations from the normal. His figures for the analyses are shown in Table XXXVIII.

Table XXXVIII. *Analysis of milk from tuberculous cows (Storch).*

	K_2O	Na_2O	CaO	MgO	P_2O_5	SO_3	Cl
1	—	—	10·91	—	15·67	—	—
2	10·87	40·67	4·34	1·27	7·10	5·08	—
3	13·27	22·39	24·67	3·43	25·42	9·21	—
4							
Diseased glands	5·08	42·37	7·52	—	8·76	—	44·64
Sound glands	12·64	21·79	19·24	—	22·22	—	27·99

Herz (36) observed in the case of a cow suffering from pox a decrease in the phosphoric acid content of the milk, which fell from 0·195 per cent. at the beginning of the observation to 0·152 per cent. after 40 days. That is, the ash contained 27·10 per cent. P_2O_5 at the beginning and 19·00 per cent. after 40 days.

Cranfield and Ling (37) give details of analyses of the milk from a cow which gave abnormal milk over three lactations. At first the cow appeared to be perfectly healthy with no reason for the abnormality but later she developed a hard quarter and reacted positively to the tuberculin test, though tubercle bacilli could not be demonstrated in the milk. Table XXXIX shows the results

Table XXXIX. *Percentage of ash, lime, and phosphoric acid in the milk of an abnormal cow (Cranfield and Ling).*

		Ash	CaO	P_2O_5	Ratio CaO/P_2O_5
1924 lactation. 33 samples, second month to end of lactation	Max.	0·88	0·178	0·215	1·52
	Min.	0·67	0·092	0·121	0·78
	Av.	0·77	0·134	0·169	1·26
1925 lactation. 114 samples, complete lactation	Max.	0·82	0·192	0·237	1·65
	Min.	0·68	0·100	0·108	0·99
	Av.	0·77	0·145	0·180	1·20

obtained for two lactations, the maximum, minimum and average for each constituent analysed being given. It will be noticed that there are very considerable fluctuations for all constituents. Perhaps the most striking is the value of the ratio P_2O_5/CaO which shows a range from 0·78 to 1·52 in the first lactation and from 0·99 to 1·65 in the second. The authors state that this ratio fluctuated considerably during the first 4 months, became steady later and finally, during the last 4 months, fell to a value approximating to unity. On two occasions the percentage of lime actually exceeded the percentage of P_2O_5—a marked abnormality. The mean value of the ratio is rather lower than that for normal mixed milk. In both lactations the insoluble ash fell steadily throughout the period while soluble ash did the opposite. Compared with mean results for mixed milk this milk gave a much higher soluble ash and a correspondingly lower insoluble ash.

Koestler's (101) investigations indicated that abnormalities in secretion were accompanied by changes in composition which followed a general rule. Thus

the albumin, globulin, chlorine, sodium and sulphate were increased while the lactose, potassium, magnesium, calcium and phosphate were decreased. The ratio of chlorine to lactose is particularly interesting. For milk from normal cows this ratio is fairly constant whereas for abnormal milk it becomes high. Thus the "chlorine-lactose number" (obtained by multiplying by 100 the ratio of percentage chlorine to percentage lactose) may be used as an index to the presence of abnormal milk. For normal milks it should be between 2·0 and 3·5.

Influence of time of milking. Published data as regards mineral constituents appear to be lacking. Eckles and Shaw (93) investigated the influence of time of milking on the protein, fat, lactose, etc., but secured no data for the mineral constituents.

The citric acid content of milk[1].

A search of the literature concerning the influence of citric acid in milk reveals firstly the important effects ascribed to citric acid, secondly, the inaccuracy of all the methods available for determining this substance, and thirdly, the extremely high range of variation exhibited in the figures for analysis under apparently normal conditions. For these reasons the subject of citric acid is considered by itself and the following is a résumé of the methods of analysis with their limits of accuracy, the values obtained for the quantity in milk by different investigators and the range of variation under different conditions.

Methods of analysis.

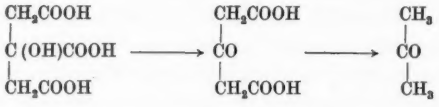
The methods of analysis may be divided into two types: (1) those depending on the estimation of insoluble salts of citric acid with metals, (2) those depending on the oxidation of citric acid to acetone-dicarboxylic acid or acetone, which may themselves be estimated by precipitation.

As regards the insoluble salts of citric acid those of calcium and barium are fairly insoluble in water and their solubility decreases with rise of temperature. Thus Camp (74) claimed to obtain a precipitate from a 0·25 per cent. citric acid solution by autoclaving at 15 lb. pressure for 20 minutes. Similarly Leighton and Mudge (75) found that the endothermic reaction which accompanies the phenomenon of heat coagulation in milk is due to the precipitation of the phosphates and citrates of calcium and magnesium. Calcium citrate is quantitatively insoluble in 50 per cent. alcohol and estimation may be made by this means provided no other insoluble salts are formed. In the presence of oxalic, lactic, and malic acids, etc., it is useless.

Of the methods of estimation depending on the oxidation of citric acid there are three which may be reviewed with interest. Careful treatment in slightly

[1] The literature on this subject was compiled by Dr N. C. Wright, to whom the author is greatly indebted.

acid solution oxidises citric acid first to acetone-dicarboxylic acid and then to acetone—thus:



(1) *Beau's method* (76), *based on Denigès' reaction.* In the presence of mercuric sulphate citric acid gives, on oxidation with permanganate, a white precipitate of a mercury-acetone-dicarboxylic acid complex of constant composition. This reaction was first observed by Denigès (77), who applied it to the detection of citric acid. The reaction is not given by malic, tartaric, succinic, or lactic acids and though oxalic acid gives a white precipitate with the reagent before the permanganate is added this may be filtered off and does not interfere with the method. It is doubtful, however, whether the reaction is really quantitative since it has been shown by Wohlk that acetone-dicarboxylic acid breaks down slowly to acetone and carbon dioxide, even at 0° C.

The method applied to the estimation of citric acid in milk, as given by Sommer and Hart (78) is as follows:

The casein is first precipitated with a dilute solution of mercuric sulphate so that the clear serum contains citric acid and an excess of the reagent. The serum is then heated and a solution of potassium permanganate added slowly. A precipitate is formed which is filtered off when cold and may be either weighed direct or titrated with KCN and $AgNO_3$, using KI as indicator. The citric acid is calculated by means of a known factor.

This method has been used by Sommer and Hart (*loc. cit.*), Salant and Wise (79), and Sherwood and Hammer (80) and is claimed to give more accurate results than those based on Stahre's reaction (81).

(2) *Method based on Stahre's reaction.* In this method citric acid is oxidised by dilute permanganate in the presence of bromine, when the water-insoluble pentabromacetone is formed. Full details are given by Kunz (82) and Hartman and Hillig (83).

Objections to the method are (1) the formation of pentabromacetone as an oil instead of a crystalline precipitate, (2) the relatively high solubility of the precipitate, (3) the loss due to its volatility when moist. Hartman and Hillig made exhaustive studies of the optimum conditions necessary and claim considerable accuracy. It should be noted, however, that the method is still open to the same objection as was instanced for the first method, namely, the doubtful quantitative production of acetone dicarboxylic acid.

(3) *Method based on the production of acetone.* Pratt (84), recognising the danger of decomposition of the dicarboxylic acid, oxidised citric acid to acetone which he distilled into a receiver containing Denigès' reagent, with which it forms a precipitate. He claimed to obtain satisfactory results but Willoman (85) was unable to secure concordant results with the method.

It may be seen from this brief discussion that the methods of estimation of citric acid are not very satisfactory.

Occurrence of citric acid in milk.

The following is a summary of the values obtained by different workers for the citric acid content of milk:

Observers	Basis of method	No. of samples	Citric acid (%)		
			Max.	Min.	Mean
Kunz(82)	Stahre	5	0·198	0·155	0·177
Bosworth and Prucha(86)	Denigès	2	0·224	0·203	0·213
Hess, *et al.*(55)	Ba salt	2 (1)	0·130	0·080	0·105
Supplee and Bellis(87)	Stahre	17	0·182	0·121	0·145
Sommer and Hart(78)	Denigès	16	0·248	0·191	0·231
Sherwood and Hammer(80)	Denigès	335	0·330	0·070	0·180
Kieferle, *et al.*(88)	Stahre	104 (2)	0·400	0·200 (3)	0·270
Scheibe(89)	Own	32	0·298	0·082	0·132
Steuart(90)	Denigès and Stahre	8 (4)	—	—	0·150

(1) Composite samples, each of 5 cows.
(2) From four quarters of 26 cows.
(3) One determination 0·12, others above 0·20.
(4) Dried milks recalculated to liquid milks.

It may be seen from these results that there are wide variations in the citric acid content of milk. The maximum is 0·40 per cent., the minimum 0·07 per cent., and the mean is approximately 0·18 per cent. The unreliable character of the methods of analysis is no doubt one factor responsible for differences in the determinations and another factor is very probably the difference in the ages of the samples used, for it has been shown by Bosworth and Prucha (91), and Van Slyke and Bosworth (63) that *B. lactis aerogenes* ferments citric acid and a similar effect has been shown by Hammer (102) for *S. citrovorus* and *S. paracitrovorus*, so that during the natural souring of milk the citric acid tends to decrease.

In view of the wide variations in results and of the unsatisfactory methods of analysis it is well to point out here that there is quite definite evidence that citric acid does occur in milk. Thus Henkel (92) isolated and identified this substance, using as a means of identification (1) the elementary composition of the crystals, (2) correct titration value with NaOH, (3) correct values of calcium content of calcium salt, and theoretical water content, (4) correct melting point, (5) solubility of the crystals in ordinary solvents, and (6) positive test with the Sabanin-Laskowski reaction. Sommer and Hart (78) have since confirmed this by similar work. There is therefore no reason to doubt that citric acid does occur in milk.

Sherwood and Hammer (80) carried out a thorough examination of 335 samples of milk from cows in all stages of lactation, of different breeds, and on various feeds. They concluded that the citric acid content is not affected by breed, period of lactation, or change from a ration of concentrates plus ensilage to pasture. Considerable variations occurred in successive milkings of individual cows, which makes it doubtful whether any of the conclusions made

about the citric acid content of milk can be relied upon at all. The following data are taken from their results:

Cow	*a*	*b*	*c*	*d*	*e*
Day 1, p.m.	0·13	0·21	0·19	0·21	0·26 %
Day 2, p.m.	0·33	0·10	0·15	0·20	0·17 %

These authors tried both the Stahre and Denigès methods and concluded that Beau's method (based on Denigès' reaction) gave the most consistent results, but they do not give the limits of accuracy for either method. It is to be noted that they show a variation of 150 per cent. in the quantity of citric acid secreted by a cow over a period of 24 hours—a variation out of all proportion to the differences exhibited by other constituents. These facts must be borne in mind when considering the claims of other investigators that the citric acid content varies in this way or in that way with stated conditions, for it appears quite probable that the daily fluctuations are large enough to mask any such effect.

Scheibe(89) using goats, stated that the citric acid in the milk was not obtained either from the citric acid or other organic acids in the feed for the following reasons: (1) Citric acid is found in human milk and this can hardly have its origin in hay or roots. (2) Doses of citric acid up to 40 g. per day did not increase the amount in the milk. (3) Feeding with wheat or bread, which do not themselves contain citric acid, still gave the normal amount of citric acid in the milk. (4) Starvation or feeding a limited supply of food also gave the normal amount. Considering the extent of the variations found by other workers we might be justified in enquiring what exactly Scheibe considered to be the "normal amount" of citric acid in milk—a point of some interest since three of his conclusions are apparently based on that estimate.

Hess, Unger and Supplee(55) claimed that a change from winter to summer feeding increases the citric acid content of the milk. Their results are shown in Table XXXVI, and as previously explained the analyses are for composite samples of five cows over a whole day's milking. However, their conclusions are based on the results of one analysis and therefore carry little conviction, particularly in view of the doubtful character of the citric acid determinations.

Supplee and Bellis(87) carried out a more carefully controlled experiment to determine the effect of summer pasture on the citric acid content of milk. Their results are shown below:

		Winter ration		Summer ration	
Herd	Cow No.	Citric acid (%)	Feed	Citric acid (%)	Feed
1	1	0·173	Hay, distiller's grains,	0·174	Fresh grass only
	2	0·121	corn stover, ensilage,	0·114	
	3	0·182	molasses	0·156	
2	4	0·145	Hay, straw, cottonseed	—	,,
	5	0·155	meal	0·148	
	6	0·106		0·130	
3	7	0·139	Hay, oil meal, corn	0·164	,,
	8	0·119	meal, bran	0·138	
	9	0·139		0·160	

The authors concluded (1) that there was a wide variation in the citric acid content of the milk of different cows receiving the same feed, (2) that the evidence of a variation in the citric acid content due to different feeds is not conclusive, (3) that comparison of summer and winter feedings showed a significant difference only in the case of herd 3, where pasture cows gave a uniformly higher citric acid content. However, these conclusions are also based on single analyses of composite samples in each case.

Kieferle, Schwaibold and Hackman(88) compared the citric acid content of samples of milk obtained from the individual quarters of the udders of 26 cows. With one exception values ranged between 0·20 and 0·40 per cent. There was a smaller variation between samples from different quarters of the same cow than between those from different cows (0·07 per cent. in the former (with one exception) and 0·20 per cent. in the latter) so that there seems to be a measure of agreement between milk samples secreted simultaneously by an individual cow.

The importance of citric acid in milk has been demonstrated, particularly from the point of view of evaporated milk, beyond all doubt and it is therefore distinctly depressing to find that data concerning the quantities in milk, their fluctuations and the causes for these fluctuations are so unreliable and conflicting. However, one definite conclusion emerges from this review of the literature—that is, that the question of citric acid in milk must be considered far more accurately and far more comprehensively than has been the case hitherto if we are to arrive at any certain knowledge in this connection. A more reliable method of estimation is one essential so that we can at least be certain that any differences observed are due to actual differences in content and not due to inaccuracies in the determination. Apart from that consideration we must know what the normal range of variation in this constituent is for individual cows and for different cows under the same conditions, how it fluctuates with lactation and what is the effect of change of feeding on the quantity secreted in the milk. For this purpose a number of very carefully controlled experiments are necessary and analyses must be made on both individual and composite samples—and of course in duplicate.

The importance of the mineral constituents and citric acid in dairy problems.

Heat coagulation and alcohol test. One of the most important influences of the mineral constituents and citric acid is in connection with the heat coagulation of evaporated milk. The final stage in the manufacture of this product consists in "sterilising" it by heating in closed containers to a temperature of about 115° C. for several minutes, the main object of this treatment being destruction of micro-organisms. In addition the milk should coagulate to form a tender jelly or "liver" which on subsequent shaking should yield a smooth and creamy product of sufficiently high viscosity to prevent the separation of fat. Different samples of milk differ considerably in the readiness with which

they coagulate, some requiring such a high temperature that the product is darkened in colour, while others form such a thick curd at the temperature required for sterilisation that they are rendered unsaleable.

It was shown by Sommer and Hart(30) that the factor controlling the temperature of heat coagulation was the balance of the calcium, magnesium, phosphate and citrate. Thus addition of very small quantities of soluble calcium or magnesium salts prevented the coagulation of some samples of milk at a certain temperature, while addition of soluble phosphate or citrate prevented the coagulation of others. A proper balance of these four constituents is necessary for stability.

Generally speaking evaporated milk contains a slight excess of calcium over the amount required for the correct balance and in consequence addition of 4 to 10 oz. of sodium phosphate per 1000 lb. of evaporated milk is commonly practised, the exact amount to be added to each batch being first determined by means of a "pilot" steriliser.

The same factors were shown by Sommer and Binney(94) to influence the coagulation of milk in the alcohol test, such small additions as 0·0112 per cent. CaO or 0·0040 per cent. MgO sufficing to induce a positive test in a milk which was originally negative. Moreover, these authors claimed that feeding calcium carbonate to cows produced a definite change in the alcohol test of the milk. Three cows were used and the time of the experiment divided into five periods. During the initial period of 10 days no calcium carbonate was fed, the milk was in every case negative to the alcohol test and required small quantities of calcium acetate to make it just give a positive reaction. In the second period of 11 days, 50 g. of calcium carbonate per head per day were fed, the milk finally became positive and required the addition of small quantities of potassium phosphate to make it just give a negative reaction. In the third period of 9 days 100 g. of calcium carbonate were fed and the milk became more and more positive, as evidenced by the increasing amount of potassium phosphate required to make it just react negatively. In the fourth period of 6 days 200 g. of calcium carbonate were fed and the milk became still more strongly positive. Finally, during a period of 8 days no calcium carbonate was fed, the milk became less and less positive and finally gave a negative reaction. The authors concluded that these results demonstrated the importance of feeding in determining the salt balance of the milk, though the differences were too small to be detected by chemical analysis.

Some light is thrown on the mechanism controlling the effect of certain salts on coagulation of milk by a series of papers by Ganguly, Dhar and coworkers(95–99). These authors found that colloids can be divided into two distinct classes: (1) those which adsorb only oppositely charged ions and therefore behave normally on coagulation by mixtures of electrolytes and follow the general dilution rule—*i.e.* that dilution decreases the amount of electrolyte necessary for coagulation, (2) those which, in addition to adsorbing oppositely charged ions, adsorb appreciably ions of the same charge. These show marked

"ionic antagonism" when coagulated by mixtures of electrolytes and do not follow the dilution rule. Adsorption of the same charged ion is the primary factor for this class of colloid and therefore additions of small quantities of an electrolyte tend to increase the repelling force between the particles and thus decrease the viscosity and increase the stability.

Milk was shown by these authors to belong to the second class of colloids and to be negatively charged. In consequence diluted milk is more stable than concentrated milk to the coagulating action of small quantities of electrolytes such as sodium tartrate or acetate because of the adsorption of negative ions. Thus according to these workers the stabilising of milk by the addition of small quantities of sodium citrate is not due to the removal of the precipitating calcium ions but is due to adsorption by the milk sol of negatively charged citrate ions and OH′ ions derived by hydrolysis.

It was also found that gelatin sensitised milk and rendered it more easily coagulable by salts. Though there is no direct experimental evidence it seems likely that albumin has the same effect and that the removal of the albumin during preheating of milk explains the increased stability to heat of the subsequently evaporated product.

Nutrition.

That the mineral constituents of milk play an important part in the nutrition of the suckling is a well recognised fact. In view of the wide variation in mineral content which this review of the literature emphasises it becomes highly important to discover exactly what factors are responsible. So far experiments have mainly demonstrated the apparently uncontrollable spontaneity with which fluctuations occur. Further and very accurately controlled experiments are necessary to discover the influence of the cow's feed, over a long period, on the mineral content of the milk.

Keeping quality.

The keeping quality of milk may be defined as the time required for natural souring at a definite temperature to produce sufficient acidity to coagulate it. The average *p*H of fresh milk is approximately 6·6 and the *p*H at which it coagulates is approximately 4·7. Milk with a relatively high mineral salt content shows a correspondingly high buffer effect and therefore the amount of acid required to reduce the *p*H from 6·6 to 4·7 is appreciably higher. In other words milk with a high mineral salt content has a superior keeping quality.

Acknowledgments.

The author wishes to express his thanks to Dr N. C. Wright for much helpful advice and criticism and to Miss D. Knight, Librarian, National Institute for Research in Dairying, and her assistants for considerable help in securing the literature.

REFERENCES.

(1) Richmond (1920). *Dairy Chemistry.*
(2) Babcock, Leach (1920). *Food Inspection and Analysis.*
(3) Orla-Jensen, Porcher and Chevallier (1923). *Le Lait*, **3**, 97.
(4) Deysher, associates of L. A. Rogers (1928). *Fundamentals of Dairy Science.*
(5) Porcher and Chevallier (1923). *Le Lait*, **3**, 97.
(6) Jordan, Hart and Patten (1906). *Amer. J. Physiol.* **16**, 268.
(7) Forbes and Keith (1914). *Ohio Agric. Expt. Sta. Tech. Bul.* **5**.
(8) Lenstrup (1926). *J. Biol. Chem.* **70**, 193.
(9) Van Slyke and Bosworth (1915). *Ibid.* **20**, 135.
(10) Wright and Papish (1929). *Science*, **69**, 78.
(11) Supplee and Bellis (1922). *J. Dairy Sci.* **5**, 455.
(12) Quam and Hellwig (1928). *J. Biol. Chem.* **78**, 681.
(13) Quam, Soloman and Hellwig (1928). *Ind. and Eng. Chem.* **20**, 930.
(14) King and Etzel (1927). *Ibid.* **19**, 1004.
(15) Supplee and Bellis (1922). *J. Dairy Sci.* **5**, 455.
(16) Rice and Miscall (1923). *Ibid.* **6**, 261.
(17) Trunz (1903). *Zeitsch. f. physiol. Chem.* **40**, 263.
(18) Nottbohm and Dörr (1914). *Zeitsch. f. Nahr. Genussm.* **28**, 417.
(19) Hammond and Hawk (1917). *J. Agric. Sci.* **8**, 139.
(20) Taylor and Husband (1922). *Ibid.* **12**, 111.
(21) Mattick and Hallett (1929). *J. Dairy Res.* **1**, 35.
(22) Grimmer (1926). *Lehrbuch der Chemie und Physiologie der Milch.*
(23) Soxhlet (1878). *Bericht über die Arbeiten der landwirtsch. Versuchs-Station*, Wien, p. 151.
(24) Katagama (1908). *Die landwirtsch. Versuchs-Station*, **69**, 342.
(25) Forbes and Beigle (1916). *Ohio Agric. Expt. Sta. Bul.* **295**.
(26) Schrodt and Hansen (1885). *Die landwirtsch. Versuchs-Station*, **31**, 55.
(27) Meigs, Turner, *et al.* (1926). *J. Agric. Res.* **32**, 833.
(28) Taken from Porcher and Chevallier (1923). *Le Lait*, **3**, 97.
(29) Schepang (1917). Dissert. Leipzig.
(30) Sommer and Hart (1926). *Wis. Agric. Expt. Sta. Res. Bul.* **67**.
(31) Cranfield, Griffiths and Ling (1927). *J. Agric. Sci.* **17**, 72.
(32) Bögold and Stein (1890). *Maelkeritidende*, p. 493.
(33) Hashimoto (1903). *J. Sapporo Agric. Coll.* **2**, 1.
(34) Schrodt (1887–8). *Jahresbericht des Milchwirtsch. Institutes, Kiel.*
(35) Storch. Cited by König (1903). *Zusammensetzung der Nahrungs- und Genussmittel*, 4 Aufl. **1**.
(36) Herz (1893). *Milchzeitung*, **22**, 348.
(37) Cranfield and Ling (1929). *J. Agric. Sci.* **19**, 491.
(38) Crichton (1930). *J. Dairy Res.* **2**, 1.
(39) Weiske (1871). *Z. f. Biol.* **7**, 179, 333; *J. f. Landwirt.* **21**, 139.
(40) von Wendt (1909). *Chem. Soc. J.* **96**, 164.
(41) Neumann (1894). *Milchzeitung.* **22**, 701.
(42) Hart, McCollum and Humphrey (1909). *Amer. J. Physiol.* **24**, 86.
(43) Becker, Eckles and Palmer (1927). *J. Dairy Sci.* **10**, 169.
(44) Zaykowsky (1926). *Biochem. Zeitsch.* **169**, 67.
(45) Lauder and Fagan (1914). *Proc. Roy. Soc. Edinburgh*, **35**, 195.
(46) Beach and Turner (1904). *Storrs Agr. Exp. Sta. Rept.* p. 150.
(47) Sommer and Hart (1921). *J. Dairy Sci.* **4**, 7.
(48) Duncombe (1924). *Ibid.* **7**, 86.
(49) Forbes, Beigle, *et al.* (1917). *Ohio Agric. Expt. Sta. Bul.* **308**.
(50) Mattick and Wright (1925). *Biochem. J.* **19**, 915.
(51) Mattick (1928). *Ibid.* **22**, 144.

(52) SHEEHY and SENIOR (1929). *Ibid.* **23**, 898.
(53) DAVIES and PROVAN (1928). *Welsh J. Agric.* **4**, 114.
(54) ORR (1929). *Trans. Highland and Agric. Soc. of Scotland*, **41**, 99.
(55) HESS, UNGER and SUPPLEE (1920). *J. Biol. Chem.* **45**, 229.
(56) PROKS (1928). *Le Lait*, **8**, 553.
(57) BENTON (1929). *J. Dairy Sci.* **12**, 481.
(58) SOMMER (1929). *Milk Dealer*, **18**, 49.
(59) FORBES, HALVERSON, *et al.* (1918). *Ohio Agric. Expt. Sta. Bul.* **330**.
(60) SHEEHY (1921). *Biochem. J.* **15**, 703.
(61) LENSTRUP (1926). *J. Biol. Chem.* **70**, 193.
(62) VAN SLYKE and BOSWORTH (1915). *Ibid.* **20**, 135.
(63) —— —— (1916). *Ibid.* **24**, 199.
(64) BRIGGS and LAPHAM (1902). *U.S. Dept. Agric. Bur. of Soils Bul.* **19**.
(65) RUPP (1913). *U.S. Dept. Agric. Bur. An. Ind. Bul.* **166**.
(66) PALMER and RICHARDSON (1925). *Colloid Symposium Monograph*, **3**, 112.
(67) GYÖRGY (1923). *Biochem. Zeitsch.* **142**, 1.
(68) GREENWALD (1913). *J. Biol. Chem.* **14**, 369; (1915) **21**, 29; (1916) **25**, 431.
(69) FEIGL (1917). *Biochem. Zeitsch.* **81**, 380.
(70) IVERSEN (1920). *Ibid.* **104**, 22 and **109**, 211.
(71) GREGERSON (1907). *Zeitsch. f. physiol. Chem.* **53**, 453.
(72) WARDLAW (1914). *J. Roy. Soc. N.S.W.* **48**, 253.
(73) MATTICK (1929). *J. Agric. Sci.* **19**, 452.
(74) CAMP (1923). *Ann. Missouri Bot. Gardens*, **10**, 213.
(75) LEIGHTON and MUDGE (1923). *J. Biol. Chem.* **56**, 53.
(76) BEAU (1904). *Rev. gén. du Lait*, p. 386.
(77) DENIGÈS (1900). *Comptes Rend.* **130**, 32.
(78) SOMMER and HART (1918). *J. Biol. Chem.* **35**, 313.
(79) SALANT and WISE (1916). *Ibid.* **28**, 27.
(80) SHERWOOD and HAMMER (1926). *Iowa Sta. Coll. Res. Bul.* **90**.
(81) STAHRE (1895). *Nord. Farm. Tidskrift*, **2**, 141.
(82) KUNZ (1915). *Die landwirtsch. Versuchs-Station*, **69**, 342.
(83) HARTMAN and HILLIG (1927). *J. Ass. Off. Agric. Chem.* **10**, 264.
(84) PRATT (1912). *U.S. Dept. Agric. Bur. Chem.* Circ. 88.
(85) WILLOMAN (1916). *J. Amer. Chem. Soc.* **38**, 2193.
(86) BOSWORTH and PRUCHA (1910). *J. Biol. Chem.* **8**, 479.
(87) SUPPLEE and BELLIS (1921). *Ibid.* **48**, 453.
(88) KIEFERLE, SCHWAIBOLD and HACKMAN (1925). *Z. f. physiol. Chem.* **145**, 18.
(89) SCHEIBE (1891). *Die landwirtsch. Versuchs-Station*, **39**, 153.
(90) STEUART (1924). *Analyst*, **49**, 465.
(91) BOSWORTH and PRUCHA (1910). *J. Biol. Chem.* **8**, 479.
(92) HENKEL (1891). *Die landwirtsch. Versuchs-Station*, **39**, 143.
(93) ECKLES and SHAW (1913). *U.S. Dept. Agric., Bur. An. Ind. Bul.* **157**.
(94) SOMMER and BINNEY (1923). *J. Dairy Sci.* **6**, 176.
(95) GANGULY and DHAR (1922). *J. Phys. Chem.* **26**, 701.
(96) SEN, GANGULY and DHAR (1924). *Ibid.* **28**, 313.
(97) GHOSH and DHAR (1925). *Ibid.* **29**, 435.
(98) —— —— (1927). *Ibid.* **31**, 649.
(99) DHAR and PRAKASH (1929). *Ibid.* **23**, 459.
(100) HART and ANDREWS (1903). *N.Y. Agric. Expt. Sta. Bul.* **238**.
(101) KOESTLER (1920). *Mitt. Lebensm. Hyg.* **11**, 154.
(102) HAMMER (1920). *Iowa Agric. Exp. Sta. Res. Bull.* **63**.

(*MS. received for publication August* 17, 1931.)

26. THE EFFECT OF NIGHT ON MILK PRODUCTION

By K. W. D. CAMPBELL, M.C., B.Sc. (Agric.) (Durham), M.Sc. (Reading).

(With 6 Figures.)

Introduction.

It is frequently stated that the long interval between the afternoon and morning milking is largely responsible for low fat percentages in morning milk. One publication (1) remarks "that as far as the percentage of fat is concerned the intervals between milkings constitute the most important of the known factors associated with variations in the composition of milk." This latter statement may be regarded as less committal than the former.

In the case of the former statement what precisely is meant? Is it implied that the length of that interval is primarily responsible for the low fats or that factors operating during that particular period of the day regardless of the length of that interval are responsible for the low fats?

The matter is regarded of some importance for two reasons:

(1) If the low fats are incidental to the long interval and the real cause of the trouble is the factors operating during that interval regardless of its length, then we must isolate and find a measure of the effect of those factors that the farmer who is compelled to adopt a long night interval may be advised accordingly.

(2) A great deal still remains to be learnt of the factors causing variation of the fat and solids-not-fat of milk. To speak in general terms of an interval being responsible for a given result is to lose sight of the fact that one factor or a small group of factors operating during that interval may be the primary cause. Not until the presence of these factors is recognised and they are then isolated can their effect be properly studied.

Object.

Campbell (2) has shown that when twelve Shorthorn cows were milked at three 8-hour intervals throughout their lactations, individual cows produced milk nearly 12 per cent. of the total of which were below 3 per cent. The same work showed also greater milk yield at the morning milking than at either of the three milkings per day and at the same time a lower fat percentage when the bulk milk of all cows in the trial was considered.

Following this work he milked two cows at 16 and 8-hour and $14\frac{1}{2}$ and $9\frac{1}{2}$-hour intervals, but with the long interval in each case from morning to night (3).

The morning and night milk yields of these two cows did not show the same relationship as that stated by Ingle (4) to apply to cows milked at the given

intervals but with the long interval during the night. A corresponding dissimilarity applied in the case of the fat percentages.

The object of the work herein reported was then to show more clearly that night itself has a definite effect on the yield and quality of milk.

METHOD.

Five cows and a heifer were included in the trial. Four of the cows and the heifer came into the trial 4 days after calving and one cow 7 days after calving. All were milked twice daily at 15 and 9-hour intervals, but the position of the long interval was changed in the case of each cow as shown by the curves. Samples of milk were taken from each cow at each milking and tested for fat by the Gerber method. Each cow had one milker only throughout the trial except on about six occasions when students had to be allowed to milk.

The work started on October 7, 1930, and finished on March 29, 1931.

Three cows and the heifer started the trial by being milked at 6.30 a.m. and 9.30 p.m. After periods varying from 6 to 10 weeks their milking hours became 6.30 a.m. and 3.30 p.m. Two were kept for periods of 5 and 7 weeks on these hours before being changed again to 6.30 a.m. and 9.30 p.m. The other two of this quartet were not changed back to the 6.30 a.m. and 9.30 p.m. milking hours. The two remaining cows started the trial by being milked for several weeks at 6.30 a.m. and 3.30 p.m., then were changed for 4 weeks to 6.30 a.m. and 9.30 p.m., and finally changed again to 6.30 a.m. and 3.30 p.m.

Each cow was kept in entirely for parts of the trial and also allowed out for morning grazing for other parts of the trial.

EXPLANATION.

The following is a key to the figures:

The two top curves of each figure show daily milk yield and daily fat yield.

The perpendicular lines divide the lactation concerned into abnormal and normal periods. Abnormal means that during the specified time the cows were milked at 6.30 a.m. and 9.30 p.m., that is, the long interval was during the day and the short during the night. Normal means that the cows were milked at 6.30 a.m. and 3.30 p.m.—the length of the intervals being the same in both cases, but the long and short intervals changing their relative positions.

Each abnormal and normal period is divided into further periods marked "In" and "Out."

"In" means that the cows were housed day and night, not being allowed out even during cleaning time, and "Out" means that they were out from about 9 a.m. until 2.15 p.m. but were in during the rest of the day.

The following explains the curves at the bottom of each figure:

In each figure a straight line AB is shown which represents the yield of milk, fat and fat percentage produced daily after the short interval.

If these yields are regarded as unity, the other curves in the figures show the relative yields after the long interval.

This method was adopted to show diagrammatically the proportion between morning and evening yield of milk, yield of fat and fat per cent. during normal and abnormal milking hour periods.

Observations.

The following points are worthy of notice:

(1) The daily milk yield and fat yield curves are normal and show no marked decrease or increase at times of change from normal to abnormal—or *vice versa*—milking hours.

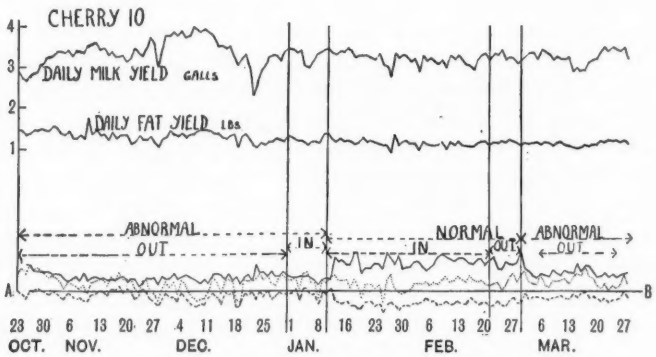


Fig. 1.

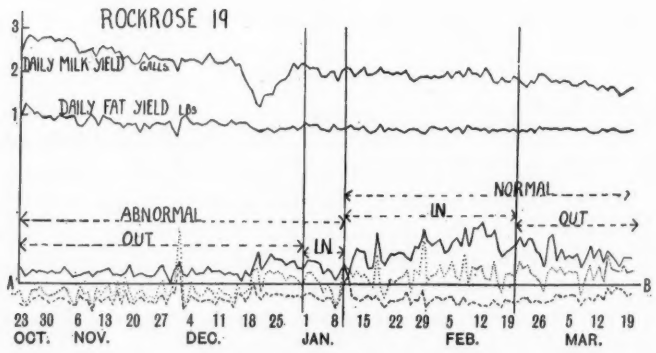


Fig. 2.

(2) The effect of the change of milking hours on proportionate yields is seen almost immediately in the case of most of the cows.

(3) Each cow produced more milk and fat after the long interval during the first few weeks of her lactation than she produced at a later stage of her lactation after the same interval, regardless of the position of the long interval.

(4) All the cows produced a bigger percentage of their day's yield of milk after the long interval when that interval was from night to morning than when it was from morning to night. The curves clearly show this to be the case

throughout each cow's stay in the trial and Tables I and II provide a measure of this increased percentage.

(5) Table II shows that by virtue of this lower milk yield after the long interval from morning to night the fat per cent. of the milk of Cherry, Rockrose 9, Fillpail and Duchess after that long interval was higher than after the long interval from night to morning.

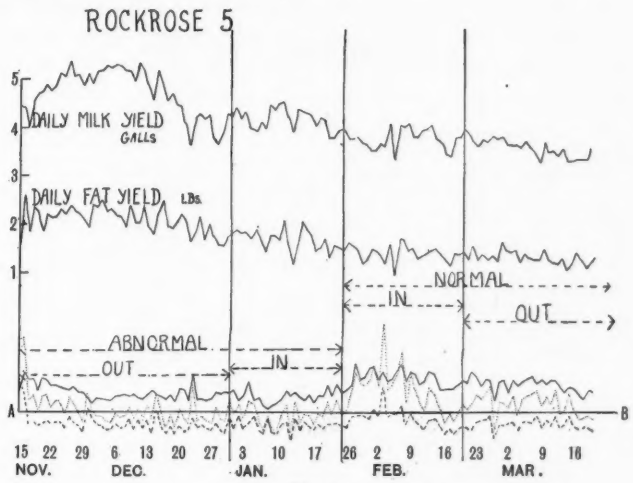


Fig. 3.

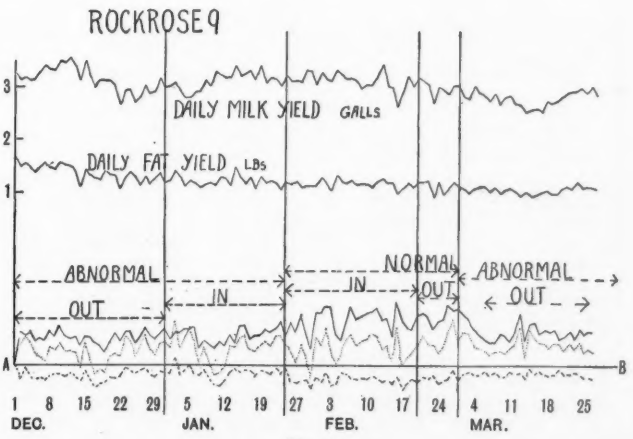


Fig. 4.

(6) The curves show that Fillpail and Duchess produced more fat after the long interval when it was from morning to night than when it was from night to morning, so that their milk at night after the long interval had a higher fat per cent. than in the morning after a long interval by virtue of this increased

fat production as well as by virtue of the reduced milk yield. Table I shows that when comparing the two normal milking periods of these two cows with the one abnormal period actually more fat was produced during the period stated as shown by the curves.

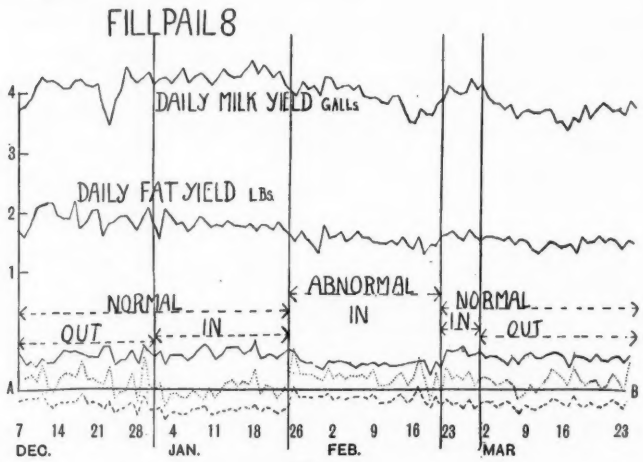


Fig. 5.

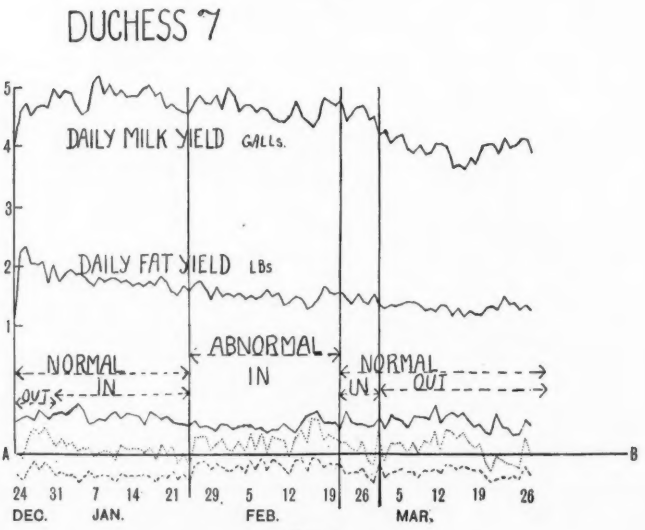


Fig. 6.

The figures in Tables I and II do not show up Rockrose 5 and Rockrose 19 to advantage in the matter of their fat yield.

They have both behaved in an obviously abnormal manner, so that too much value cannot be attached to them. The figures of Rockrose 19 in Table I

under ratio of milk yield to milk yield will explain to some extent her failure to conform to the four cows mentioned above in the matter of fat yield.

The curves show that Rockrose 5 behaved in an unusual way for about 3 weeks after the change from abnormal to normal milking hours. During that 3 weeks her morning fat per cent.—that is, after the long interval—was on two

Table I. *Showing ratio of milk yield to milk yield, fat per cent. to fat per cent. and fat weight to fat weight of all cows individually as between short and long intervals. Where one cow has two abnormal milking hour periods these are taken together and similarly with normal milking hour periods.*

	Ratio of milk yield to milk yield. Milking hours		Ratio of fat % to fat %. Milking hours		Ratio of fat yield to fat yield. Milking hours	
	6.30 a.m. & 3.30 p.m.	6.30 a.m. & 9.30 p.m.	6.30 a.m. & 3.30 p.m.	6.30 a.m. & 9.30 p.m.	6.30 a.m. & 3.30 p.m.	6.30 a.m. & 9.30 p.m.
Cherry 10	1 to 1·73	1 to 1·35	1 to 0·69	1 to 0·88	1 to 1·20	1 to 1·19
Rockrose 19	1 to 1·82	1 to 1·29	1 to 0·68	1 to 0·68	1 to 1·23	1 to 0·88
Rockrose 5	1 to 1·63	1 to 1·36	1 to 0·77	1 to 0·73	1 to 1·26	1 to 1·00
Rockrose 9	1 to 1·87	1 to 1·57	1 to 0·73	1 to 0·84	1 to 1·37	1 to 1·32
Fillpail 8	1 to 1·56	1 to 1·41	1 to 0·70	1 to 0·82	1 to 1·09	1 to 1·19
Duchess 7	1 to 1·61	1 to 1·49	1 to 0·68	1 to 0·82	1 to 1·10	1 to 1·26

Table II.

		Yield of milk after long interval as percentage of day's yield. Milking hours		Yield of fat after long interval as percentage of day's yield. Milking hours		Fat % after long interval when that after short interval is unity. Milking hours	
	Period of lactation	6 a.m. & 3 p.m.	6 a.m. & 9 p.m.	6 a.m. & 3 p.m.	6 a.m. & 9 p.m.	6 a.m. & 3 p.m.	6 a.m. & 9 p.m.
Cherry 10	First 81 days	—	56·8	—	53·95	—	0·88
	Next 49 days	63·3	—	54·7	—	0·70	—
	Next 28 days	—	59·0	—	55·7	—	0·87
Rockrose 19	First 81 days	—	56·8	—	46·85	—	0·68
	Next 68 days	64·6	—	55·2	—	0·68	—
Rockrose 5	First 72 days	—	57·7	—	49·9	—	0·73
	Next 54 days	62·09 (60·9)	—	55·8 (52·7)	—	0·77 (0·72)	—
Rockrose 9	First 56 days	—	60·4	—	55·9	—	0·83
	Next 35 days	65·0	—	57·8	—	0·73	—
	Next 28 days	—	62·9	—	59·2	—	0·85
Fillpail 8	First 49 days	61·2	—	51·7	—	0·67	—
	Next 28 days	—	58·5	—	54·5	—	0·82
	Next 34 days	60·7	—	53·2	—	0·74	—
Duchess 7	First 33 days	62·6	—	52·8	—	0·67	—
	Next 28 days	—	59·8	—	55·7	—	0·82
	Next 34 days	60·5	—	51·8	—	0·70	—

or three occasions higher than her afternoon fat per cent. The curves show this. Omitting that period of 3 weeks, however, it may be said that she conforms reasonably well in the matter of fat yield and does not prejudice the conclusion. The figures in brackets under her name in Table II relate to the last 5 weeks she was in the trial and show a tendency to conform to those of other cows in the trial.

Briefly, points (4), (5) and (6) indicate that a lower proportion of the day's milk yield and a higher proportion of the day's fat yield were produced after the long interval when it was from morning to night than when it was from night to morning. The total result of this combination of events was to give a higher fat percentage at night after a long interval than in the morning after a long interval in the case of the four cows mentioned under point (5).

Table II shows the milk yield and fat yield of each cow during each period of the trial, after the long interval, expressed as a per cent. of the day's yield. The fat per cent. after the long interval in the case of each cow is also shown when that after the short interval is unity.

The figures in Table II show the effect of advancing lactation relating to milk yield and fat yield proportions, an effect already pointed out by Bartlett (5). Nevertheless, in each succeeding period of the lactation concerned, the points enumerated above hold in a very marked manner as far as the four cows are concerned.

It will be noticed that all the cows showed a marked drop in milk yield during the 16 days ending January 7. During this period practically every cow in milk had a very violent attack of scour that lasted for 2, and in some cases for 3 days. The cause of this was never ascertained, but it made its mark on milk yields. It should be noticed that Rockrose 5 dropped in milk yield at this time and never fully recovered again.

One further variation of milk yield must be noted as it applied to all the cows in the trial.

On Wednesday, March 11, a change of cake was made and some of the cows refused the new cake for a day or so. Rockrose 9 refused it completely. On Friday, March 13, a change from pulped mangolds to whole mangolds became necessary owing to an accident to the pulper earlier in the week. These factors together appear to have resulted in a marked drop in yield of all cows in the herd. It must be noticed that the drop in the case of Cherry 10 and Rockrose 9 corresponds with a temporary breakdown in the relationship previously held, between morning and evening milk yield and fat yield.

Discussion.

The object of this work has been concisely stated above. It is proposed to analyse some of the factors operating during the night because until these factors are isolated and studied it will be impossible to do more than is shown by this work, namely, that night itself has an effect on milk production.

The following conditions exist between the night and morning milking on a large number of farms in ordinary practice:

(1) Darkness, in the winter for from 11 to 13 hours, in the summer for about 5 hours (south of England).

(2) In winter, where cows are housed at night, a period of quiet broken only by "haying down" about 9 p.m.

(3) In summer, a period of quiet and rest limited possibly by the hours of darkness. In any case there will likely be more movement during the summer night than during the winter night, in addition to the walk to and from the field at night and morning. This last factor must not be overlooked with regard to its effect on the general health of cattle. Where thrice daily milking is practised, cattle may be actually on the road for from 1½ to 2½ hours in every 24. Food will be required to make good this waste of energy. If milk yield is maintained and more food, grazing, is taken in, the period of rest during the night may be shorter even than the hours of darkness.

(4) On some farms a scarcity of water during winter nights, a scarcity emphasised in some cases by the dry nature of winter keep and the closeness of the atmosphere.

(5) In the summer, if there is no shortage of water in the fields, this factor will not operate at night or during the day.

(6) A longer interval between morning and night milking and, in the winter, a slightly longer interval between meals than occurs in either case during the day.

(7) Last, but not necessarily least, the question of temperature has to be considered. In a badly ventilated cowhouse in the winter the atmosphere may become humid and Bender (6) has found that humidity not temperature appeared to affect milk production. Hays (7) concludes as a result of his own work that temperature is a major factor in the seasonal variation of the per cent. of fat in cow's milk. Weaver and Matthews (8) conclude that butterfat tests were lower with high outside and inside temperatures and add that there were indications that variations in the fat test were more closely related to variations of outside temperature. Campbell (9) has shown that there may be differences of over 35° F. between day and night temperatures outside in the spring and summer months and differences as between the temperature outside during the day and inside during the night ranging from 18° to 35° F. and expresses the opinion that temperature variation per 24 hours is a fruitful cause of fluctuation of milk yield and fat percentage.

If further work shows that temperature variation during any 24 hours produces a physiological reaction on cows during the night, then we may have isolated one of the factors, operative during winter and summer, that will function to produce the results indicated in the following summary.

SUMMARY.

Curves are produced to show the proportions between morning and evening yield of milk, yield of fat, and fat per cent. in the case of individual cows milked alternatively at 6 a.m. and 9 p.m. and again at 6.30 a.m. and 3.30 p.m. In each case the intervals between milkings were 15 and 9 hours.

It is seen that when the 15-hour interval is between night and morning milkings as specified, a bigger proportion of milk is produced at the morning

milking than is produced at night when the same interval is between morning and night milkings. The curves and Tables I and II show that this is quite clearly the case with the six cows in the trial.

It is apparent that, as far as winter conditions are concerned, night itself, or factors operating at night tend to high milk production of low fat content.

REFERENCES.

(1) Ministry of Agriculture (1929). *Misc. Pub.* **65.**
(2) Campbell (1930). *J. Agric. Sci.* **20,** 213.
(3) —— (1930). *Ibid.* **20,** 223.
(4) Ingle (1920). *Manual of Agricultural Chemistry,* 356.
(5) Bartlett (1929). *J. Agric. Sci.* **19,** 38.
(6) Bender (1928). *New Jersey Agric. Exp. Sta. Report* (abstract from Exp. Sta. Record (1929), **61,** 765).
(7) Hays (1926). *J. Dairy Sci.* **9,** 219.
(8) Weaver and Matthews (1928). *Iowa Agric. Exp. Sta. Res. Bull.* **107.**
(9) Campbell (1931). *J. Agric. Sci.* **21,** 11.

(*MS. received for publication, June* 30, 1931.)

27. THE AUTOXIDATION OF BUTTERFAT

I. FACTORS INFLUENCING THE REACTION

By LINDSAY HEATHCOTE BRIGGS.

Dairy Research Institute, Massey Agricultural College, Palmerston North, New Zealand.

(With 3 Figures.)

Of the processes occurring in butter during cold storage, possibly the chief one is oxidation of the butterfat, resulting in the formation of tallowy flavours accompanied by bleaching. Ordinarily the oxidation may be influenced by biological action among others, but at cold storage, where this is either destroyed or kept in check, the oxidation is influenced by purely chemical factors. The following investigation was therefore made to ascertain the influence on the oxidation of butterfat of various substances associated with butter itself or during its manufacture. As oxidation occurs only very slowly at 0° the reaction was considerably hastened by working at 100°. Although the results thus obtained with butterfat at 100° are not strictly comparable with conditions affecting butter at 0° on account of the change in physical properties, they do indicate what may be expected to occur at 0° in a much diminished degree, while the method gives some idea of the relative importance of factors influencing the reaction.

In the following experiments, fresh, dry, filtered butterfat (100 g.) was heated in a flask maintained at 100° and rapidly stirred (400 r.p.m.) in an atmosphere of oxygen (Fig. 1). The flask was connected through a side piece to two gas burettes, both of which were enclosed by jackets through which water was circulated from a thermostat at 25°. Oxygen was admitted to the system, evacuated initially, through two sulphuric acid wash bottles. Differences in volume due to absorption of oxygen could be compensated simply by admitting mercury from a reservoir through a tap. The mercury seal was a modification of the usual type, where, by using only a small quantity of mercury and sliding a rubber stopper down the stem of the stirrer into the mouth of the seal, evacuation of the flask and filling with oxygen could be accomplished without further alterations to the system. The stopper was moved up the stem and more mercury added when the stirrer was set in motion.

Liquid catalysts were added as such, but solid catalysts were added in the form of dilute aqueous solutions after which the water was removed *in vacuo*. The catalysts would then be present in a very fine state of division, especially as the quantities used were in most cases only a few parts per million of butterfat.

The phenomenon of autoxidation was observed in every case, the induction periods varying from 1 to 33 hours. As the rate of absorption and the volume of oxygen absorbed were of the same order even when catalysts were used, except for the first few, experiments were carried out for a time only sufficient to indicate the initial rate of oxidation.

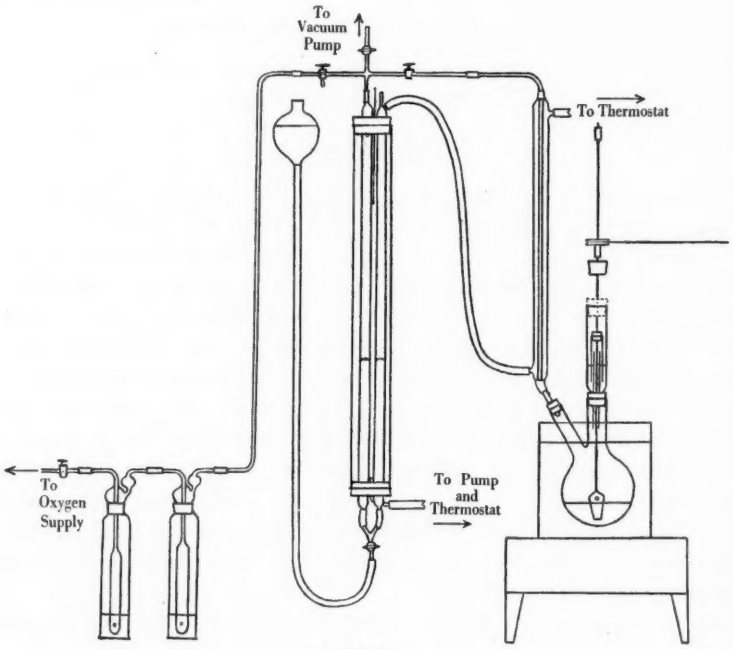


Fig. 1.

Series I. Controls.

Exp.	1	2	3	4	5	6	7	8
Induction period (hr.)	15	14¼	14¼	15	15¼	14¼	14¼	14¼

This series consists of experiments carried out at intervals during 3 weeks on eight different portions from the same sample of butterfat, with a view to ascertaining whether any considerable change took place on keeping. Fig. 2 represents graphically the results in this series where oxidation was carried to completion, from which it may be seen that there is a maximum divergence of 1 hour in the induction periods. In other series, however, greater and unexplained divergences were sometimes obtained, but in the majority of cases these did not exceed 2 hours. A substance was therefore assumed to have no influence unless it altered the induction period by 3 hours. The induction period of 38 different control experiments throughout the year averaged 16 hours, much longer periods than those experienced by Smith and Wood (2 hr.)(1), and

Holm and Greenbank (2 to 4 hr.)(2), as well as those recorded for all other natural fats. Although the amount of oxygen absorbed varied in different samples, for which no suitable explanation can be given, the rate of absorption was very similar even where catalysts were used, as can be seen in Fig. 3. The

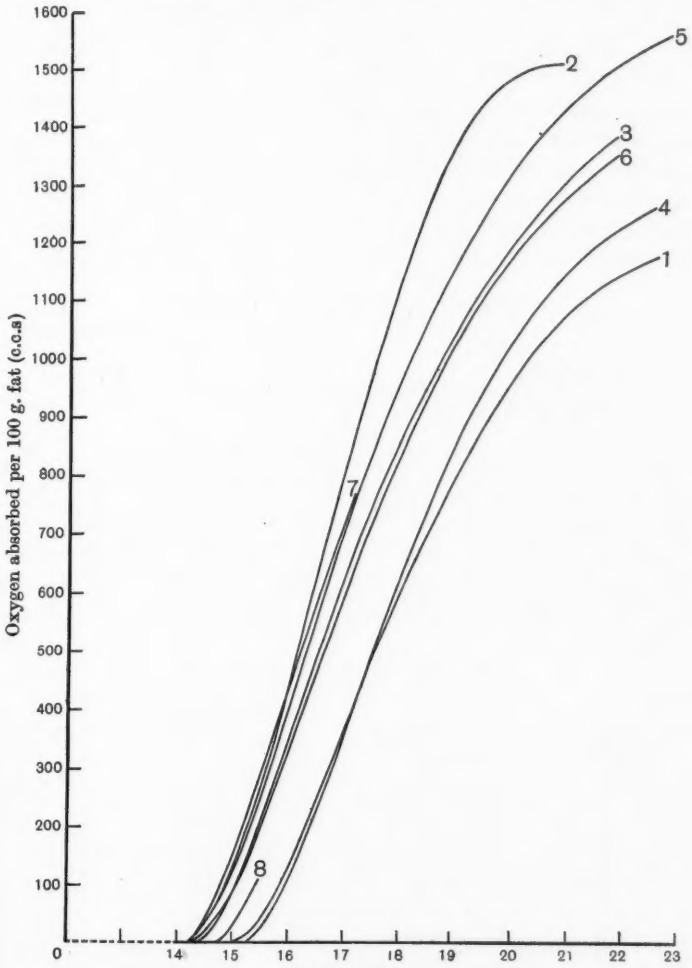


Fig. 2.

rate of absorption was approximately twelve times greater than that observed by Holm and Greenbank and Smith and Wood. No irregularities in the rate of absorption of oxygen were observed similar to the case in Holm and Greenbank's experiments where "moloxides" are assumed to be formed. The experiments of this series show also that a sample of dry, filtered butterfat can be

preserved, in this case at 0°, for nearly 3 weeks without altering the induction period. Similar samples stored for 7 and 4 months at room temperature and at 0° respectively also showed no appreciable change in the induction period.

Series II. The influence of moisture.

	Induction period (hr.)		
Control exps.	A 16¼, 16½	B 14½	C 19¾, 14
Exps. with moisture	16	14¾	21½

The experiments with moisture have been carried out on dry butterfat in an atmosphere of oxygen saturated with water vapour. Holm and Greenbank have shown that moisture lengthens the period of induction at 95° by 1 hour and also that samples of butter preserved at different humidities showed tallowy changes greatest in those stored at the lowest humidities. The above results show, however, that humidity has no effect on the induction period.

Series III. The influence of light.

	Induction period (hr.)
Control exps.	av. 17½
Exp. exposed to ultra-violet light of a mercury arc lamp at 16 in. distance	5½

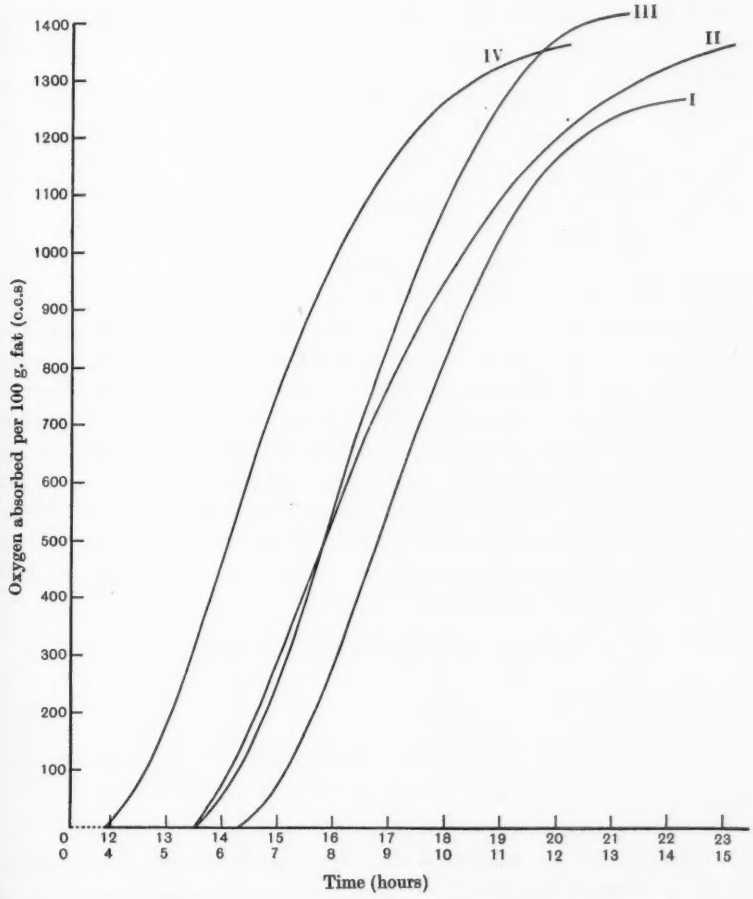
Frazier (3) has shown that sunlight exerts a strong influence on milk, producing tallowy flavours, and that the defect is restricted to the fat portion. Whitehead (4) has found a similar change in the reduction of methylene blue by sterile milk in sunlight which occurs in whole milk but not in separated milk.

The above experiments show that ultra-violet light has a considerable influence in hastening the oxidation, reducing the period of induction to one-third.

Series IV. The influence of metals.

Exp.	Catalyst	Concentration of catalyst	Induction period (hr.)
A 1	Control (*a*)	—	21½
2	,, (*b*)	—	19¾
3	Sodium vanadate	$4 : 10^6$	2½
4	Copper lactate	$4 : 10^6$	3
5	Iron lactate	$4 : 10^6$	15
6	Nickel sulphate (*a*)	$4 : 10^6$	18
7	,, (*b*)	$4 : 10^6$	19
8	Zinc lactate	$4 : 10^6$	25
B 1	Control	—	14½
2	Copper lactate	$1 : 10^6$	3¾
3	,,	$1 : 10 \times 10^6$	8
4	,,	$1 : 10 \times 10^6$	8¼
5	,,	$1 : 20 \times 10^6$	10
6	,,	$1 : 40 \times 10^6$	11
C 1	Control	—	25
2	Copper lactate Sodium pyrophosphate	$1 : 10^6$ $2 : 10^6$	3½
3	Copper lactate Sodium pyrophosphate	$1 : 10^6$ $2 : 10^6$	1¾
D 1, 2, 3	Controls	—	av. 17½
4	Iron lactate	$4 : 10^6$	13½
5	,,	$7 : 10^6$	12½

It has long been known that traces of iron and copper may act as catalysts in oxidation. The above experiments show, however, the relative intensity of these two metals as catalysts as well as the action of other metals. Thus copper and vanadium salts to the extent of 4 parts per 1,000,000 of butterfat exert a



I. Control. II. Ultra-violet light.
III. Ferrous lactate. IV. Lactic acid.
(Lower figures in time axis refer to ultra-violet light curve.)

Fig. 3.

remarkably strong influence reducing the induction period from 20½ hr. to 3 and 2½ hr. respectively, while copper lactate to the extent of 1 part in 40,000,000 of butterfat still has an appreciable action in hastening the change. Series C represents attempts to diminish the catalytic action by forming a

complex copper salt, but apparently there is no protective action with pyrophosphate.

Iron has a strong catalytic action but not nearly so pronounced as that of copper. Series D is included here for it shows the remarkable action of iron even when the lactate is added as single crystals.

Nickel salts appear to exert only a slight pro-oxidative influence on the autoxidation, while zinc lactate in similar proportion appears to have a slight retarding action.

Series V. The influence of peroxides.

Exp.	Catalyst	%	Induction period (hr.)
A 1–4	Controls	—	av. $15\frac{1}{2}$
5	Hydrogen peroxide	0·2	$8\frac{1}{2}$
6	Oxidised fat	1	$8\frac{1}{2}$
B 1	Control	—	$9\frac{3}{4}$
2	Oxidised fat (heated at 100°/10 mm.)	0·1	9

It has been indicated that fats on oxidation give rise to peroxides and even to hydrogen peroxide (5). Holm and Greenbank found that fresh butterfat when mixed with old butterfat had a shorter induction period. From the above experimental results it may be seen that both hydrogen peroxide and oxidised fat have an equal and intense pro-oxidative influence on the reaction. The active catalyst in oxidised fat is stable under the ordinary experimental conditions of heating at 100°/760 mm. but is destroyed by heating at 100°/10 mm. as is shown in the experiment of Series B where oxidised fat was added before the usual period of evacuation for 1 hour at 100°/10 mm. preceding the oxidation, the method used in drying the butterfat.

Series VI. The influence of acidity.

Exp.	Catalyst	%	Induction period (hr.)
A 1–3	Controls	—	av. $15\frac{3}{4}$
4	Lactic acid	0·1	$11\frac{3}{4}$
B 1–3	Controls	—	av. $17\frac{1}{4}$
4	Lactic acid	0·1	12

The experiments of this series show that lactic acid (0·1 per cent.) has a marked influence in hastening oxidation which agrees with the views of Rogers and his associates (6) and Dyer (7) that butter made from high acid cream has inferior keeping qualities to butter made from low acid cream.

Series VII. The influence of triolein.

Exp.	Catalyst	%	Induction period (hr.)
1	Control	—	$9\frac{3}{4}$
2	Triolein	0·1	9

When added in concentration of 0·1 per cent. triolein had no effect on the oxidation. Bevis (8) has found that oleic acid when added to beef fat promotes

rancidity. Oleic acid is more unstable than triolein, but probably does not exist as such in butter. The author's result is in accordance with the fact that fats poor in oleic acid often become tallowy more quickly than fats rich in oleic acid and often more quickly than oleic acid and triolein, which indicates that tallowiness depends not on the concentration of unsaturated compounds or on the availability of oxygen but on the susceptibility of the fat to oxidation regulated by other factors.

Series VIII. The influence of glycerol.

Exp.	Catalyst	%	Induction period (hr.)
A 1	Control	—	$8\frac{1}{2}$
2	Glycerol	0·1	12
B 1–4	Controls	—	$15\frac{3}{4}$
5	Glycerol	1	12

Hunziker and Hosman(9) ascribe tallowiness in part to glycollic acid derived from the oxidation of glycerol or lactose in the presence of metallic catalysts. Bevis found that added glycerol appeared to have little influence on the oxidation of beef fat as well as in the formation of substances giving the Kreis test. The evidence of this series is conflicting. Whereas glycerol in concentration of 1 per cent. appears to hasten the oxidation, in concentration of 0·1 per cent. it appears to exert a protective action. The explanation may be that glycerol has either no appreciable action (considering both the results within the experimental limit) or that in different concentrations it has different actions.

Series IX. The influence of lactose.

Exp.	Catalyst	%	Induction period (hr.)
1	Control	—	$9\frac{3}{4}$
2	Lactose	0·1	$9\frac{1}{2}$

Hunziker and Hosman have observed that in acid or neutral butter lactose had no effect but produced tallowy flavours in alkaline butter, a condition which is, however, not a natural one. Lactose in the author's experiment had no effect on a sample which would have a very low acid value.

Series X. The influence of pasteurisation.

Treatment	Induction period (hr.)	
Unpasteurised	A $12\frac{1}{2}$, 14, $13\frac{1}{2}$	B 14, 20, av. 17
Pasteurised	$14\frac{1}{2}$, $14\frac{3}{4}$	$16\frac{1}{2}$, $16\frac{1}{4}$

Although the chemical condition of milk and cream is altered during the process of pasteurisation, the above experiments show that no property affecting the induction period of the butterfat is altered during the flash pasteurisation of the cream from which the butter was made.

Series XI. The influence of iodine.

Exp.	Catalyst	%	Induction period (hr.)
1, 2	Controls	—	$16\frac{1}{4}$, $16\frac{1}{2}$
3	Iodine	$1:10^6$	$14\frac{3}{4}$
4	,,	$10:10^6$	$13\frac{1}{2}$
5	Potassium iodide	$4:10^6$	$17\frac{1}{2}$

Iodine has been shown to have antioxygenic properties (10), but in the above experiments the influence of iodine is negligible or, if anything, hastens the oxidation. It was thought possible that potassium iodide added would destroy any peroxide initially formed and thus have an antioxygenic effect but it appears to have little action in that direction.

Series XII. The influence of curd.

Exp.	Catalyst	Induction period (hr.)
A 1	Control	$17\frac{3}{4}$
2	With curd	$30\frac{1}{4}$
B 1, 2	Controls	av. $20\frac{1}{2}$
3	With curd	$32\frac{1}{2}$
C 1	Control	30
2	With curd	$27\frac{3}{4}$

Owing to some decomposition of curd at 100° the above results are not comparable with the influence of curd at 0°. The results are interesting however, for they show that curd on heating at 100° produces a strong anticatalytic effect. This is very apparent in two cases but in the third case it appears to have little action. The anticatalytic action has probably arisen from the decomposition of nitrogenous material with formation of an amino compound which belongs to the general class of antioxidants.

In another series, a dry, filtered butterfat giving an induction period of 33 hours was noticed to have a turbid appearance due, it was assumed, to colloidal casein. On further treatment by heating at 100°/10 mm. for 1 hour and filtering it gave an induction period of 25 hours. Further heating at 100°/10 mm. in other cases produced no such change. The second sample still appeared slightly turbid, but no differences could be detected in their nitrogen values owing to the very small amount of curd present. These experiments are significant however, in that it is possible for colloidal casein to be held by dry butterfat and so exert an antioxygenic effect. If the amount varies, as is presumed to be the case here, so the induction period varies, which may account for the differences observed in control experiments prepared at different times.

Summary.

The influence of a number of factors on the autoxidation of butterfat has been studied. Metallic catalysts have been found to exert a great effect in hastening the reaction, their intensity being in the following decreasing order:

sodium vanadate, copper lactate, iron lactate, nickel sulphate. Zinc lactate has a slight retarding action.

Ultra-violet light, hydrogen peroxide and fat peroxides have a strong pro-oxidative effect and to a less extent lactic acid. Curd exerts an antioxygenic effect, while humidity, glycerol, triolein, lactose, iodine, potassium iodide and the effect of pasteurisation have little or no influence.

The author wishes to thank the Auckland University College Council and Sir James Gunson for a Sir James Gunson Scholarship during the tenure of which this research was undertaken, Dr F. H. McDowall for his helpful interest in the work, and the Dairy Research Institute (N.Z.) for permission to work in their laboratories.

REFERENCES.

(1) SMITH and WOOD (1926). *Ind. Eng. Chem.* **18**, 691.
(2) HOLM and GREENBANK (1923). *Ibid.* **15**, 1051; (1924) **16**, 518, 598; (1925) **17**, 625. HOLM, GREENBANK and DEYSHER (1927). *Ibid.* **19**, 156.
(3) FRAZIER (1928). *J. Dairy Sci.* **11**, 375.
(4) WHITEHEAD (1930). *Biochem. J.* **24**, 579.
(5) STUTZ, NELSON and SCHMUTZ (1925). *Ind. Eng. Chem.* **17**, 1138.
(6) ROGERS and GRAY (1909). *U. S. Bur. of Animal Ind. Bull.* **114**; ROGERS, THOMPSON and KEITHLEY (1912). *Ibid.* **148**, 27.
(7) DYER (1916). *J. Agric. Res.* **6**, 927.
(8) BEVIS (1923). *J. Soc. Chem. Ind.* **42**, 417 T.
(9) HUNZIKER and HOSMAN (1917). *J. Dairy Sci.* **1**, 320.
(10) MOUREU and DUFRAISSE (1924). *Compt. rend.* **178**, 824.

(*MS. received for publication, March* 31, 1931.)

28. THE AUTOXIDATION OF BUTTERFAT

II. COMPARISON OF TESTS FOR DETECTING OXIDATION CHANGES

By LINDSAY HEATHCOTE BRIGGS.

Dairy Research Institute, Massey Agricultural College, Palmerston North, New Zealand.

(With 5 Figures.)

In Part I (1) the influence of a number of factors on the autoxidation of butterfat was investigated. It was considered of interest, however, to study the oxidation itself more fully to gain some idea of the changes taking place, and also to study certain reactions for detecting such changes.

In tallowy butter as the result of oxidation, the following differences in properties can be observed: (1) the amount of unsaturated compounds is decreased; (2) the peroxide content is increased; (3) the amount of free acid is increased; (4) the colour is bleached. Secondary changes occur simultaneously and result in the formation of aldehydes, ketones, etc.

The change in some of the above properties has been traced quantitatively during the autoxidation of butterfat by measuring the direct absorption of oxygen and estimating the iodine, acid and peroxide values at intervals.

The results are given in the following table and are expressed graphically in Figs. 1, 2, 3 and 4, representing the rise in the acid value, the absorption of oxygen by the butterfat, the peroxide value and the decrease in the iodine value respectively.

Time (hr.)	0	14	15	16	17	18	19	20	21	22	23
Acid value	0·85	0·88	1·0	1·06	1·35	2·18	2·64	3·18	3·9	4·09	4·01
Peroxide value	0	0·37	0·42	0·49	0·58	0·55	0·71	0·73	0·75	0·85	0·85
Iodine value	40·2	40·2	39·9	38·65	37·2	35·8	34·65	33·7	33·4	32·5	33·4
Oxygen equivalent to difference in iodine value*	—	—	28	150	294	425	535	626	659	737	659
Oxygen absorbed per 100 g. fat*	0	0	9	160	363	560	700	790	840	875	895

Induction period 14¾ hr.

* Calculated at 25°.

On comparing these results the following points may be noticed:

(1) The acid, iodine and peroxide values remain practically unchanged during the induction period.

(2) The change in the acid value does not show a close relation to the absorption of oxygen, the curve of the graph showing a distinct lag indicating

that the production of acid is a secondary process. The acid value therefore does not give a satisfactory means for detecting the progress of oxidation.

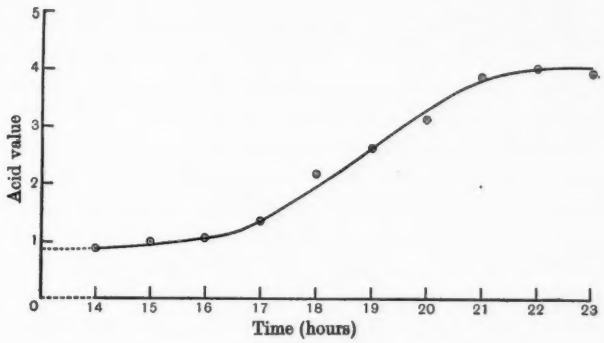


Fig. 1.

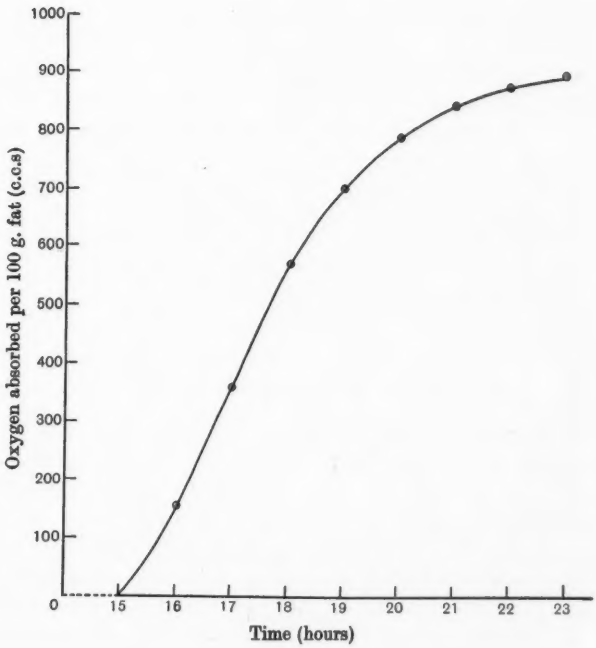


Fig. 2.

(3) The peroxide values determined in the ordinary way (2) also do not correspond well with the absorption of oxygen, notwithstanding the fact that, in the latter cases especially, the values should be greater, for some of the iodine liberated in the determination would be absorbed by unsaturated com-

pounds. The limit of error in the determination also is high. The estimation of the peroxide value is hence unsuitable for ascertaining the degree of oxidation.

(4) The iodine value decreases more nearly in proportion to the absorption of oxygen. The discrepancy as well as the peroxide values shows that the oxidation proceeds further than the oleic peroxide stage. The iodine values, however,

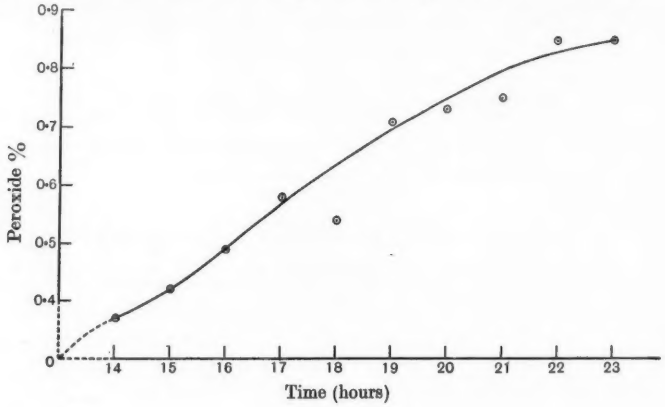


Fig. 3.

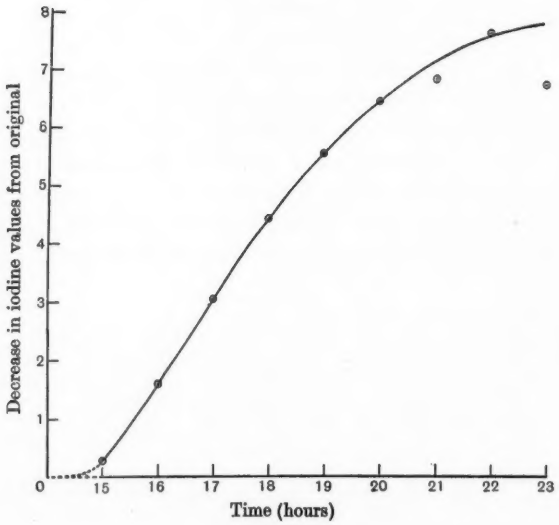


Fig. 4.

should be higher, for the action of acetic acid used in the determination would tend to destroy any peroxide formed and liberate more unsaturated compounds. Although the determination of the iodine value gives no indication of any changes during the induction period, it does give a fairly satisfactory guide to the progress of later autoxidation.

THE KREIS TEST.

This has been followed quantitatively by Holm and Greenbank (3) who have found that the colour intensity produced in the Kreis test is directly proportional to the amount of oxygen absorbed. In the author's investigations this has not been followed quantitatively but it has been found that to the naked eye the intensity of the colour produced in the Kreis test increases with the volume of oxygen absorbed. In Holm and Greenbank's experiments, however, the period of induction was not given, but is assumed to be 2–3 hours on the basis of similar experiments carried out by them (3). The period of induction in the author's experiment was about 15 hours and interesting results during this period were obtained. Kreis tests were applied to samples of butterfat withdrawn every hour during the autoxidation. Although there was no appreciable absorption of oxygen during the period of induction, at the end of 3 hours the butterfat withdrawn gave a very faint Kreis test. Further samples gave tests gradually increasing in intensity until at the end of 8 hours quite a distinct positive reaction was given. This increasing intensity was steadily maintained until the end of the induction period, after which a distinct change was noticed. The sample withdrawn at the end of 15 hours gave a Kreis test of much greater intensity which gradually increased again with further samples until the final test after 23 hours when the colour was a very dark red.

	Time (hr.)	Reaction
Induction period	0	Yellow—negative
	3	Yellow—very slight trace of pink
	5	Yellow—slight trace of pink
	8	Quite distinct pink
	13	Red coloration
	15	Deep red—contrast to previous test
	23	Very deep red

COMPARISON OF TESTS FOR OXIDATION IN FATS.

These interesting results with the Kreis test led the author to make further investigations to compare this test with other sensitive reactions for the detection of small changes in fats.

Many tests involving the detection of peroxides have been introduced from time to time such as the haemoglobin and guaiacol tests but they have not found widespread use. Besides the Kreis test, the Fellenberg or Schiff's reaction seems to be used extensively. Both these tests rely on the detection of aldehydes. Other reactions, however, relying on the same principle have been brought forward but have only a small or specific use.

Recently the Department of Scientific and Industrial Research (England) (4) have introduced a rapid test for the detection of small quantities of peroxides by the liberation of iodine from potassium iodide followed by titration with very dilute standard thiosulphate solution. This method enables a quantitative estimation to be made.

It was thought of interest, therefore, to compare the results of tests carried out by the Kreis, Fellenberg and this latter method during the induction period in the autoxidation of butterfat. The results obtained are interesting not only in the comparison of the three tests, but also in indicating the changes occurring in fats during the induction period.

The sample of dry, filtered butterfat used had been prepared from pure Jersey butter and had been kept in the refrigerator for 4 months. The induction periods of the original and preserved samples were, however, identical, showing that dry, filtered butterfat can be preserved for several months without change in its susceptibility to oxidation. During the experiment, Kreis, Fellenberg and peroxide tests were carried out on weighed samples withdrawn hourly. A sample was also kept to determine any changes in colour due to bleaching and any appearance of tallowy odours, comparisons being made with an untreated control sample.

The induction period of the experiments quoted below was 18¼ hours.

Kreis tests.

Time (hr.)	Reaction
0	Yellow—negative
8	Yellow—negative
9	Very slight trace of pink in acid layer
10	Slight trace of pink in acid layer
11	Distinct pinkish tinge
12	Stronger pinkish tinge
17	Stronger pinkish tinge
18	Red coloration—much stronger than previous test
19	Very red coloration—much more intense than test at 18 hr.

Fellenberg test.

Time (hr.)	Reaction	Colour of ethereal layer
0	Negative—no colour in aqueous portion	Light brown
5	Positive—bluish colour in liquid interface	Light brown
10	,,	Lighter shade
14	,,	Colour gradually fading
18	,,	Colourless
19	Intense purple colour in aqueous portion	Bluish tint

Colour and odour of butterfat.

Time (hr.)	Odour	Colour
0	Fresh, pleasant, characteristic smell of good butter	Normal
15	,,	,,
16	Slightly stale, the odour of fresh butter still present	,,
17	,,	,,
18	Tallowy odour	Slightly bleached
19	Intense tallowy odour, odour of fresh butter absent	Entirely bleached

Peroxide values.

These results are given in the following table and are represented graphically in Fig. 5.

Time (hr.)	0	1	2	3	4	5	6	7	8	9
Peroxide content (% × 10^4)	—	5·0	3·8	7·1	8·8	10·2	10·1	11·0	14·5	15·0
Time (hr.)	10	11	12	13	14	15	16	17	18	19
Peroxide content (% × 10^4)	18·0	19·2	22·3	21·1	23·7	32·8	30·6	33·1	47·8	349·2

Induction period 18½ hr.

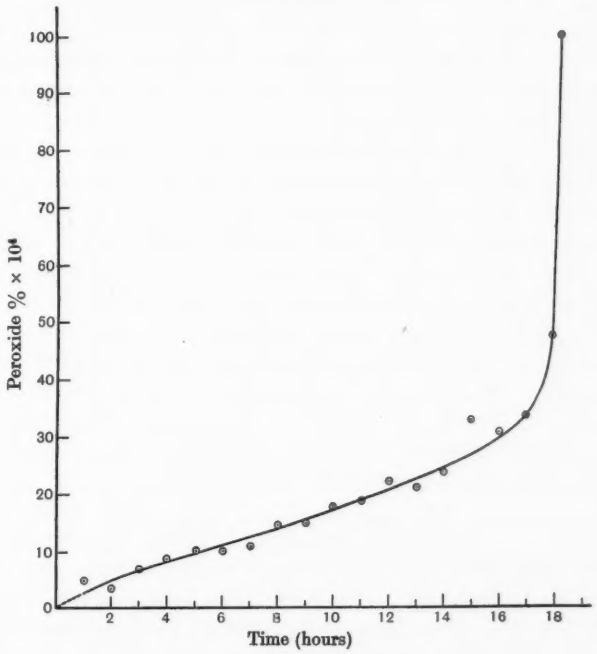


Fig. 5.

This graph shows that although there is no appreciable absorption of oxygen during the induction period, changes are occurring in the fat resulting in the formation of small amounts of peroxides and that the estimation of the peroxide value gives the best indication as to the extent of the change. Moreover, the method has the advantage of being used quantitatively while the time of operation also is only a few minutes. By this method the amount of peroxide formed is estimated while both the Kreis and the Fellenberg tests are dependent on the formation of aldehydes, the result of a secondary reaction. For this reason both the Kreis and Fellenberg reactions are only given several hours after the commencement of the experiment, whereas peroxide values are

given immediately, increasing with the time of reaction at a rate which is almost linear up to the end of the induction period. The Fellenberg reaction is given at the end of 5 hours but the intensity of the colour produced does not apparently change until after the induction period. When an increase in the colour intensity is shown, the fat has already undergone changes which are easily apparent to the eye and to the sense of smell. Consequently the Fellenberg test can be used only as a qualitative but not as a quantitative test for oxidation changes.

The Kreis test is given at the end of 9 hours, but the intensity of the colour produced increases up to the end of the induction period, after which there are still greater increases of intensity during oxidation. For this reason, the Kreis test may be used in detecting the extent of oxidation changes in butter. It is worthy of note here that the Kreis test is given by butterfat during the induction period several hours before such changes as bleaching or the production of tallowy odours can be detected.

Although to the naked eye the butterfat did not appear to change in colour to any appreciable extent until 1 hour before the end of the induction period, a bleaching effect was noticed in the ethereal layer of the Fellenberg test. The colour was a light brown equally in all the first tests, but at the end of 10 hours a lighter shade was observed, the intensity of which decreased with the succeeding samples until the last but one which was quite colourless. The final sample showed a bluish tinge due to the Fellenberg reaction.

It is significant that the first positive Kreis reaction is given almost at the same time (at the end of 9 hours), as the appearance of the first trace of bleaching (as judged by the colour in the Fellenberg reaction), and that the intensity of both gradually increase from this period, pointing to some relation between the Kreis test and decoloration of the butter.

Observations on the Kreis test.

Although the Kreis test has been used for many years on account of its great value in detecting the onset of oxidation in fats, the formation of the compound coupling with phloroglucinol and hydrochloric acid to give the reaction is far from being understood while there is still doubt as to the original compound itself.

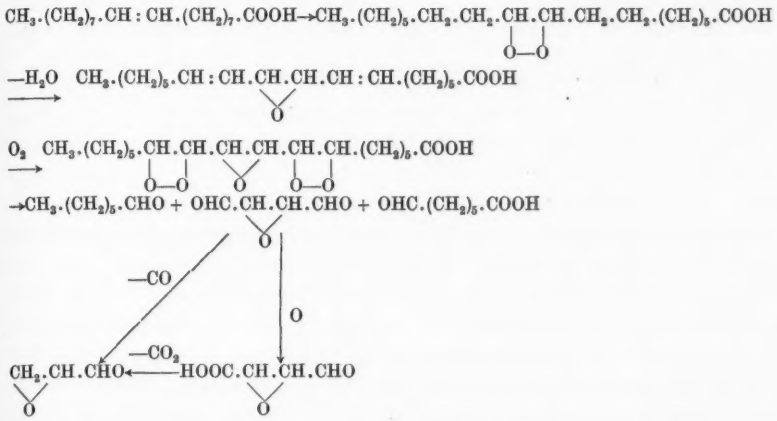
The Kreis test is given by many substances and especially by compounds occurring in essential oils, *e.g.* eugenol. Kobert (5) was able to show that only compounds containing an allyl group or a substituted allyl group

$$(—CH_2.CH:CRR')$$

are capable of forming red condensation products with phloroglucinol. Acrolein (6) gives with the Kreis test only a faint pink coloration which is intensified by the action of hydrogen peroxide.

Powick (6) by spectroscopic means identified the compound in oxidised fat giving the Kreis test as epihydrin aldehyde which he prepared for comparison by the interaction of acrolein and hydrogen peroxide. In his opinion, however,

free epihydrin aldehyde is not present in fats as such but probably exists as an acetal which liberates the free aldehyde on addition of the hydrochloric acid used in the reaction. On the basis of these conclusions he has suggested a scheme to explain the formation of epihydrin aldehyde or its precursors in the atmospheric oxidation of oleic acid.



Such a scheme, however, is most unlikely. The mechanism is most unusual while it does not explain the formation of oxido-oleic, dihydroxystearic, azelaic and nonylic acids, among the chief decomposition products of oleic acid. In his scheme also epihydrin aldehyde is formed as the result of a long series of oxidations and dehydrations.

In the author's experiments, during the induction period (18¼ hours) of practically no oxygen absorption, positive Kreis tests are given long before the end of this period. Thus it seems impossible that, on Powick's theory, a few molecules of oleic acid should be oxidised almost completely to give rise to epihydrin aldehyde while the large excess of oleic acid, more readily oxidisable than its decomposition products, should not be attacked. On these grounds, although the end products of the oxidation of oleic acid may give the Kreis reaction, it is necessary to look for some other substance capable of giving the Kreis test in the earliest stages.

If acrolein itself is assumed to be the parent substance in the production of the Kreis test, it is conceivable that it may be produced either by oxidation of glycerol set free by hydrolysis of the fat or from some unsaturated molecule. As shown by the acid values, hydrolysis of fat does not occur in the initial stages if at all. Acrolein must arise therefore by the oxidation of some unsaturated molecule. Oleic acid has been excluded for reasons stated above and likewise linoleic acid need not be considered. Salway (7) was unable to identify acrolein from oxidised oleic or linoleic acid but isolated it in the case of linolenic acid. He has shown that the decomposition of linolenic acid may occur in the following way.

Linolenic acid I. absorbs oxygen with the formation of an oxygenide (cf. Salway and Kipping (8)) and since the central point of unsaturation should be relatively stable there would be a tendency to form a dioxygenide II. This would decompose spontaneously or in the presence of moisture with formation of aldehydes including fumaraldehyde. The unknown aldehyde is unstable and immediately suffers direct disruption with the formation of acrolein and carbon monoxide or is partly oxidised with elimination of carbon dioxide and acrolein.

$$\underset{\text{I.}}{\text{R.CH:CH.CH:CH.CH:CHR'}} \rightarrow \underset{\text{II.}}{\text{R.CH.CH.CH:CH.CH.CH.R'}}$$

$$\rightarrow \text{R.CHO} + \text{OHC.CH:CH.CHO} + \text{R'CHO}$$

$$\rightarrow \text{CH}_2\text{:CH.CHO} + \text{CO}$$

or $$\text{HOOC.CH:CH.CHO} \rightarrow \text{CH}_2\text{:CH.CHO} + \text{CO}_2$$

Thus acrolein is produced by two spontaneous stages from the peroxide of linolenic acid. In conjunction with the peroxide, acrolein would thus be able to give the Kreis reaction. If linolenic acid is present in butter, it could thus be responsible for the Kreis test in the earliest stages. It is quite possible that linolenic acid is present in butter, but owing to the great analytical difficulties in the isolation it has not yet been identified. It may be mentioned that linoleic acid has only recently been identified in butter by Hilditch and Jones (9) in as much as 4 per cent.

Besides linolenic acid, it is possible also that carotene may be the precursor of the substance giving the Kreis reaction as is indicated by the bleaching of the fat occurring at approximately the same time as that at which the first Kreis test is given. Carotene has recently been shown by Karrer to have the following probable formula (10):

$$\text{C}_9\text{H}_{15}\text{—CH:CH.}\overset{\text{CH}_3}{\overset{\bullet}{\text{C}}}\text{:CH.CH:CH.}\overset{\text{CH}_3}{\overset{\bullet}{\text{C}}}\text{:CH.CH:CH.CH:}\overset{\text{CH}_3}{\overset{\bullet}{\text{C}}}\text{.CH:CH.CH:}\overset{\text{CH}_3}{\overset{\bullet}{\text{C}}}\text{.CH:CH.C}_9\text{H}_{15}$$

It is thus seen to have eleven ethylenic linkages, eight of which are conjugated. Decomposition similar to that occurring with linolenic acid would give rise to crotonaldehyde which is capable of giving a Kreis test.

The conflicting records of the action of the Kreis reagent on oleic acid and its derivatives may possibly be due to traces of these more unsaturated impurities which are very difficult to remove.

Experimental.

The autoxidation of the butterfat was carried out in the apparatus and manner described in Part I (1) except that the flask was fitted with a sampling device. During the oxidation samples were withdrawn before evacuation, after filling with oxygen and then hourly to the end of the experiment. In the first series, the acid, peroxide and iodine values were determined on each sample, while the reaction with the Kreis test was also observed, comparisons being made with untreated control samples. Allowance was made each time

for the sample withdrawn on the weight of the fat left in the flask and the corresponding velocity of oxygen uptake and total volume of oxygen absorbed. The readings were then calculated in each case for the absorption per 100 g. of fat. The acid value was determined in the usual way using as solvent an equal mixture of acid free ether and alcohol. The iodine value was determined by Wij's method while the method of Morrell and Marks (2) was used for the estimation of the peroxide value.

In the second series the Kreis and Fellenberg tests were carried out with the usual reagents. For the determination of small quantities of peroxide a modification of the method used by the Department of Scientific and Industrial Research (England) was probably used as complete details of the method were not available.

Approximately 0·7–0·8 g. of fat (warm) were weighed out into a clean medium sized test tube. The fat was dissolved in 10 c.c. of a mixture of glacial acetic acid and carbon tetrachloride (2 : 1) freed from oxidisable material by the method of Orton and Bradfield (11). A small amount of powdered potassium iodide was then added on the point of a penknife, the mixture heated to boiling for a few seconds, cooled under the tap and poured into a small titration flask containing 5 c.c. of a 5 per cent. solution of potassium iodide in water. As this operation only takes a few seconds the filling of the tube with nitrogen as recommended in the original paper was not done. The test tube was washed out with twice its volume of cold recently boiled distilled water. 1 c.c. of a freshly prepared starch solution was then added and the mixture titrated against 0·002*N* thiosulphate solution. Blank determinations were made at intervals and gave fairly consistent results, the divergences being due probably to a rather indefinite end point. The peroxide content was calculated making allowance for the blank, the value being expressed in per cent. multiplied by 10,000.

The author wishes to thank the Auckland University College Council and Sir James Gunson for a Sir James Gunson Scholarship during the tenure of which this research was undertaken, Dr F. H. McDowall for his helpful interest in the work, and the Dairy Research Institute (N.Z.) for permission to work in their laboratories.

REFERENCES.

(1) BRIGGS (1931). *J. Dairy Res.* **3**, 61.
(2) MORRELL and MARKS (1929). *Analyst*, **54**, 503.
(3) HOLM and GREENBANK (1923). *Ind. Eng. Chem.* **15**, 1051; (1924) **16**, 518, 598.
(4) Report of the Food Investigation Board for the year 1929 (1930). *Analyst*, **55**, 508.
(5) KOBERT (1907). *Zeit. Anal. Chem.* **46**, 711.
(6) POWICK (1923). *J. Agr. Res.* **26**, 323.
(7) SALWAY (1916). *J. Chem. Soc.* **109**, 138.
(8) SALWAY and KIPPING (1909). *Ibid.* **95**, 166.
(9) HILDITCH and JONES (1929). *Analyst*, **54**, 75.
(10) KARRER, HELFENSTEIN, WEHRLI, WETTSTEIN (1930). *Helv. Chim. Acta*, **13**, 1088.
(11) ORTON and BRADFIELD (1927). *J. Chem. Soc.* 983.

(*MS. received for publication, June* 17, 1931.)

29. THE EFFECT OF HEAT UPON THE RENNIN COAGULATION. II

By GEORGE M. MOIR, M.Sc., A.I.C., Ph.D.

National Institute for Research in Dairying, University of Reading.

(With 1 Figure.)

In Part I of this investigation(1) changes in the coagulability of heated milk with rennin were found to be accompanied by more or less parallel changes in the amount of nitrogen contained in the whey which was subsequently separated from the coagulum. The changes were greater when market milk (of high bacterial content) was pasteurised than when clean milk was similarly treated. The results were, however, not strictly comparable owing to the differences (both chemical and biological) of the milk which was used. In order to obtain more comparable results, experiments were planned in which the same clean milk was used throughout. This was divided into three portions, of which one (A) was pasteurised fresh; the second (B) received a small amount of starter and was pasteurised next day when it had developed considerable acidity; while the third (C) was kept under the same conditions as B, and shortly before being pasteurised its acidity was raised to approximately the same as that of B by vigorously stirring into it a small amount of strong lactic acid solution. Compared with the volumes of milk, the volumes of starter and lactic acid used were of the order 1/500 and 1/200 respectively, so that their influence upon subsequent determinations was well within experimental error. The pasteurisation was carried out in a tubular apparatus similar in every way to that previously used but considerably larger, so that the milk was in the heating chamber for 60 seconds instead of only about 25 to 30 seconds. The longer period of heating permitted more uniform control of temperature and rate of flow; it also intensified the changes produced by the heat so that clean milk pasteurised at 165° F. was much more definitely altered than when similarly treated in the small apparatus. The dimensions of the larger apparatus were as follows: length of inner, intermediate and outer tubes respectively, 4 ft., 3 ft. 9 in., 3 ft. 4 in., external diameters respectively, 1 in., 1·75 in., 2·25 in.

Rennet tests were carried out on both raw and pasteurised samples of each, and after coagulation the curd was filtered from the whey through the paper as before. Duplicate rennet tests were performed throughout for A and also for the raw and first pasteurised test of the other two portions. In Table I are set down average values, the deviation from which rarely exceeded 3 per cent. and in no case were the variations sufficiently great to modify the general

trend of the results. As mentioned previously, care was necessary in warming the milk to rennetting temperature in order to obtain the best results. The variations in the nitrogen in the whey from duplicate rennet tests were sometimes greater than were expected. The slow method of filtration through paper may have been the cause of this; in some other duplicate experiments where the whey was separated centrifugally from the curd soon after rennetting, better agreement of its nitrogen content was obtained. The total nitrogen in the whey was determined in the usual way, and in addition a measure of the non-protein nitrogen was obtained by removing the protein with Almen's tannin acid solution. This reagent contained 5 g. of nitrogen-free tannic acid dissolved in 240 c.c. of 50 per cent. alcohol, to which 10 c.c. of 25 per cent. acetic acid were subsequently added. For the analysis, 25 c.c. of whey, after dilution with exactly 50 c.c. of water, were treated with 25 c.c. of the reagent. The mixture was allowed to stand overnight before being filtered through a dry paper into a dry vessel, from which 50 c.c. of the filtrate were subsequently pippetted for Kjeldahl determination. The value thus obtained was doubled to give the non-protein nitrogen, and the very small error due to the volume of the precipitate was neglected as all the determinations were carried out in the same way. No further analyses of the whey were made since the previous experiments showed that the nitrogen content underwent the most significant variations.

DISCUSSION OF RESULTS.

Nitrogen in the whey from raw milk.

Comparison of the figures (see Table I) for the raw portion of each of the three types of milk (A, B, C) makes it clear that the increase of acidity (whether produced by bacteria or by lactic acid added to the milk) causes an increase in

Table I.

Milk	Acidity, lactic acid (%)	Whey label	Time after pasteurisation hr. min.	Time of rennet test min. sec.	Whey nitrogen (%)	Whey non-protein nitrogen (%)
Fresh	0·175	Raw	—	3 53½	0·178	0·074
A		Past. 1	30	4 14	0·175	0·072
		„ 2	1 25	4 11	0·177	0·073
		„ 3	3 30	4 17	0·173	0·072
		„ 4	6 0	4 17	0·171	0·066
Starter	0·255	Raw	—	39	0·213	0·068
B		Past. 1	30	55½	0·188	0·070
		„ 2	1 25	58	0·188	0·070
		„ 3	3 45	60	0·183	0·069
		„ 4	6 35	59½	0·183	0·068
Lactic acid	0·265	Raw	—	48½	0·185	0·073
C		Past. 1	25	54½	0·181	0·070
		„ 2	1 40	60	0·182	—
		„ 3	3 55	60	0·177	0·070
		„ 4	5 40	60	0·177	—

the amount of soluble nitrogen in the whey. Proteolysis might be suggested to explain this change but for the fact that an uncontaminated starter culture, such as was used, is generally admitted to have very little proteolytic power in

its early stages of growth. If the change were due to proteolysis, an increase in the non-protein nitrogen is to be expected instead of the slight decrease observed in the wheys from the milk treated with starter. The fact that the change is much greater in the portion treated with starter than that in which acid alone was used suggests that the bacteria play an important part. On the other hand, it must be remembered that the bacteria were producing acid slowly for about 24 hours while the lactic acid was added to the milk only about 2 hours before pasteurisation. On account of the slowness of protein reactions, this short time may have been insufficient for equilibrium to be reached. It is possible that the development of acidity causes the freeing of soluble protein (albumin or globulin) which is loosely combined with, or adsorbed upon, the fat globules (or the casein particles). The question is thus raised of the extent to which these two proteins are retained by the curd when milk is coagulated with rennin under varying conditions, and whether the whey contains these proteins in the same proportion as the original milk.

Effect of pasteurisation.

Pasteurisation produced distinct changes in all three types of milk, but most in the milk to which starter had been added. When the nitrogen content of the whey is plotted against the rennet tests (see Fig. 1), the linear relation between them again appears, in spite of one or two discrepancies. The latter are noticeable especially in A and C, and are probably due to the smallness of the changes, the determination of which would thus involve comparatively large experimental errors. Had pasteurisation been carried out at a higher temperature (*e.g.* 185° F., see Tables III and V of previous paper), such discordant results would probably have been avoided. These results taken together with those already described, leave no doubt of a relationship between the two properties under discussion. The smallness and irregularity of the variations in the non-protein nitrogen suggest that these are due to experimental error. The variations in the whey nitrogen must, therefore, be attributed to the protein constituents thereof.

SIGNIFICANCE OF THE RESULTS.

The most important fact which appears to have been established by the experiments described in this paper and the preceding one, is the correlation between the gradual increase in time of the rennet test of milk after it has been pasteurised, and the decrease in the protein nitrogen contained in the resulting whey. The experimental conditions prevented the curd itself, even in the finely divided state, from passing into the whey filtered from it. One must therefore conclude that in proportion as the milk coagulated less readily so the curd retained more and more of the soluble milk proteins, albumin or globulin,—further experiments are required to decide which. Both these proteins belong to the class of which it has been stated "the outstanding character of albumins and globulins is that they can be *coagulated by heat.*" (2) There is little accurate

knowledge available concerning the effect of heat upon the soluble milk proteins, but it is quite reasonable to expect that they will behave in much the same way as other proteins of the same type (*e.g.* egg albumin and serum albumin) which have been studied by many investigators. It has long been known that the heat coagulation of egg albumin occurs in two stages: (1) denaturation, (2) flocculation. The first of these is the more important and it can

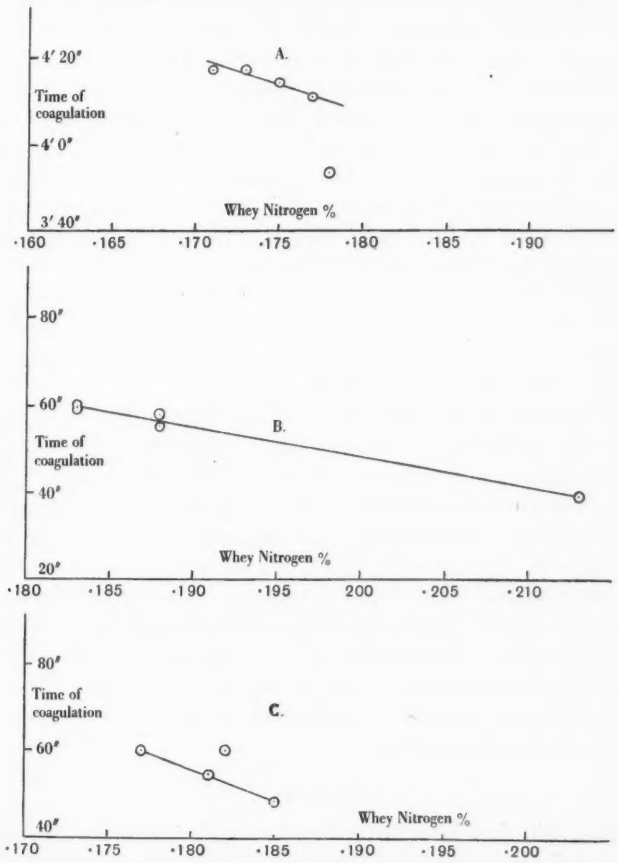


Fig. 1.

occur with little visible change, especially if the conditions (*e.g.* *p*H) are not at the optimum for the second stage. In other words, if conditions are unfavourable, flocculation or precipitation of the denatured protein may be delayed, but it must eventually occur, for the effect of denaturation is to convert the protein into a less soluble form. Such a hysteresis or lag in the completion of a protein reaction is well known, *e.g.* the slow setting of a gelatin gel after melting.

Now if the soluble protein is being flocculated or precipitated it is much more likely that it will settle upon or around other dispersed material in the solution (such as the casein or the fat globules) than that it will itself form separate particles or micelles. Palmer and Richardson(3) have refuted the theory that lactalbumin in the undenatured state exercised a "protective" effect upon the casein dispersion in milk; but it is quite possible that after denaturation (whether partial or complete) this protein, or lactoglobulin, or both, may become precipitated around the casein particles and thus "protect" them, *i.e.* prevent the free access of rennin to them so that the coagulation takes longer to occur. This idea derives support from the following statement which Whitaker, Sherman and Sharp(4) were led to make as a result of their viscosity experiments upon skim milk and whey: "The aggregates formed by heat coagulation of the whey proteins are markedly affected by the presence of the casein." Further support is provided by an experiment conducted by Zoller(5) who compared the viscosity of caustic soda solutions of casein prepared from raw milk and from the same milk after pasteurisation for 45 minutes at 65° C. The latter treatment caused an increase in viscosity of about 25 per cent. over that of the casein from the raw milk. Such increases in the viscosity of colloidal solutions are usually ascribed to increases in the particle size of the dispersed material.

In putting forward this theory to explain the results of the foregoing experiments the writer is well aware that it cannot be expected to provide a complete explanation of the decreased coagulability of heated milk with rennin. The matter is complicated by other changes produced by heat, such as the precipitation of soluble calcium, and changes in the electric charge of the casein particles(6). Nor does it explain the more rapid coagulation obtained when milk is treated with rennin immediately after pasteurisation at low temperatures—a phenomenon which is perhaps related to changes in the degree of hydration of the casein particles. Although a considerable amount of evidence has been gathered to support the theory, its full justification must await complete and thorough investigation of the effects of heat upon the individual soluble milk proteins.

Summary.

1. Clean milk has been "flash" pasteurised at 165° F. Portions of the same raw milk have been treated with small amounts respectively of starter culture and lactic acid, so as to raise the acidity, before being similarly pasteurised.

2. Comparison of the whey obtained by rennetting the three raw portions has shown that those from the acidified milk contained more soluble protein than the original.

3. The differences between the raw and pasteurised milk in their coagulation time with rennin have been found to be proportionately much greater in the acidified portions.

4. These differences in coagulation time have been shown to be correlated with decreases in the amount of soluble protein found in the whey separated from the coagulum.

5. These experimental results, together with those of other investigators, have been made the basis of a suggestion that the decreased coagulability of heated milk with rennin is partly due to flocculation of soluble milk protein upon the casein micelles.

ACKNOWLEDGMENTS.

The writer wishes to express his indebtedness to the authorities of the National Institute for Research in Dairying (University of Reading) for the facilities provided to enable the foregoing experiments to be carried out. He is particularly grateful to Capt. J. Golding and Dr E. C. V. Mattick for their interest and helpful criticism; and also to Prof. Wm. Riddet, Dairy Research Institute, N.Z., who pointed out the need for work of this nature.

REFERENCES.

(1) MOIR (1930). *J. Dairy Res.* **2**, 68.
(2) JORDAN LLOYD (1926). *Chemistry of the proteins*, p. 45. (Churchill.)
(3) PALMER and RICHARDSON (1925). *Third Colloid Symposium Monograph*, p. 121. (Chemical Catalog Co.)
(4) WHITAKER, SHERMAN and SHARP (1927). *J. Dairy Sci.* **10**, 361.
(5) ZOLLER (1921). *J. Gen. Physiol.* **3**, 649.
(6) RICHARDSON and PALMER (1929). *J. Phys. Chem.* **33**, 557.

(*MS. received for publication August*, 1930.)

30. OBSERVATIONS ON THE COPPER AND IRON CONTENT OF MILK AND OTHER DAIRY PRODUCTS

By W. L. DAVIES.

National Institute for Research in Dairying, University of Reading.

Much information has been collected in these laboratories during the past few years on the copper and iron content of milk and various dairy products. These data have resulted from work of a routine and advisory nature mostly connected with the occurrence of fat taints such as "oiliness" in milk (from commercial processing sources) and "fishiness" or "tallowiness" in butter (mostly from Dominion sources). It was considered advisable that such findings as have accumulated up to the present should be placed on permanent record.

In addition, greater significance has been placed during the past few years on the effect of small traces of heavy metals, especially iron and copper, on the dietetic value of a food or ration, particularly from the anaemia-preventing standpoint, and the effect of small traces of these metals on the partial destruction of the vitamins of foodstuffs has been investigated (Mattill(1), Hess and Unger(2)).

The analytical results have a further significance in that they show the degree of metallic contamination of milk products usually obtained under commercial conditions together with a range of figures within which attendant deterioration of the product has occurred. The scope of such data makes them as valuable as those obtained from investigations carried out under controlled laboratory conditions.

The quantitative determination of traces of iron and copper.

With the increasing study of the significance of the effects of small traces of heavy metals has developed a number of micro-methods for the estimation of iron and copper. Three reliable methods for the micro-estimation of copper have been used from time to time by the author. These were (*a*) the ferrocyanide method of Lampitt, *et al.*(3), (*b*) the xanthate method of Scott and Derby (Scott, *Standard Methods of Chemical Analysis*), and (*c*) the pyridine-thiocyanate (Biazzo) method as modified by Elvehjem and Lindow(4). The three methods involve colorimetric comparison with a colour intensity developed under identical conditions from a known amount of copper in solution and it has been found that they are, with a little practice, admirably adapted for the estimation of 0·01 mg. of copper. But owing to the importance of keeping to one method for comparative purposes more attention has been

devoted to the xanthate method. This method has been criticised in that it requires more attention in the buffering of the final acid solution, since, if the solution is too acid, a cloudiness is formed which makes colorimetric comparison impossible and there is a tendency to precipitation of the xanthate. By keeping rigidly to the conditions described for the method and adding 1 c.c. of a centrifuged freshly prepared 1 per cent. alcoholic solution of potassium ethyl xanthate, the occurrence of cloudiness was rare.

In many instances copper and iron were determined in the same ash. The metals were separated as in the xanthate method for copper, by taking advantage of the solubility of copper salts in ammonia solution. The ash was dissolved in hot 2 *N* hydrochloric acid, filtered, and the solution boiled. Excess of ammonia was added and the precipitate filtered; the precipitate was dissolved in acid and reprecipitated from boiling solution three times in succession. The filtrates were concentrated for the copper estimation and the precipitate (containing the iron) was dissolved in a small quantity of dilute hydrochloric acid and the iron estimated.

The method of estimating the iron was the thiocyanate colorimetric method. The presence of pyrophosphoric acid in milk ash usually causes a quick fading of the red colour, but this was guarded against by the use of acetone, the final solution for colorimetric comparison having an acetone content of 25 per cent. Colours obtained by this method remained permanent for at least 48 hours. The successive boiling of the acid solutions during the separation of iron and copper changed most of the pyrophosphoric acid into the orthophosphoric form. The number of successive extractions of the precipitated phosphates in the case of butter was reduced to two on account of the small amount of ash.

IGNITION OF MATERIAL. MILK, CHEESE, AND DRIED MILK PRODUCTS.

(*a*) *Milk.* One hundred ml. of milk were evaporated to dryness after the addition of a few drops of nitric acid to curdle the proteins. Ashing of the dry material was carried out at as low a temperature as possible, using almost a smoky bunsen flame burning in a wide upright asbestos pipe as an improvised muffle. The last traces of carbon were burnt off after wetting the ash with water or dilute nitric acid. Twenty to thirty g. of cheese or dried milk products were used for the ashing. Silica crucibles were mostly used for the ashing, but in a few cases large porcelain crucibles boiled in *aqua regia* were used. All used crucibles were kept immersed in *aqua regia* when not in use.

(*b*) *Butter.* One hundred g. of butter in two or three batches were melted in a crucible and the water driven off by gentle heat. The temperature was raised to the burning point of the fat, allowing the fat to burn away quietly. The charred mass was extracted with water to dissolve out the salt, as this in its molten condition occluded carbon particles and had a deteriorative effect on the crucible walls. The salt solution was filtered off and the filter paper

(iron and copper-free) returned to the crucible. The whole was ashed to a grey ash. The salt solution and acid solution of the ash were combined and the determinations proceeded with. The burning of the fat could be accelerated by letting a fine stream of air impinge on the burning fat surface.

Results of analyses.

A. *Milk.*

Analyses of a number of samples of (*a*) fresh, (*b*) pasteurised, and (*c*) sterilised milk have been made.

(*a*) *Fresh milk.* The copper content of 15 samples of fresh milk (from the Institute herd) which had been in contact with a heavily tinned milking pail only ranged from 0·15 to 0·65 parts per million and the iron content ranged from 1·50 to 2·40 p.p.m. No reason for the comparatively wide range of contents of both metals is forthcoming, as the variation was not seasonal nor was the higher copper content accompanied by a high iron content and *vice versa*.

Twelve samples of non-processed milk (except for cooling) from the south of England which did not develop "oiliness" ranged in copper content from 0·20 to 0·95 p.p.m. and from 1·60 to 2·80 p.p.m. in iron content. The range was still greater in these cases due undoubtedly to metallic contamination from utensils or coolers, but about which information was not collected. Ten samples of milk from farther afield showed yet a greater range, namely from 0·30 to 1·40 p.p.m. of copper and 1·8 to 3·6 p.p.m. of iron. (These samples came from the Midlands and Yorkshire.) The higher copper contents approached that which was found necessary usually for the development of oiliness (1·5 p.p.m. of copper as the minimum value).

These figures agree in range with those of Supplee, *et al.*(5) and Elvehjem, *et al.*(6)

(*b*) *Pasteurised milk.* The copper content of 39 samples of pasteurised milk ranged from 0·4 to 4·0 p.p.m., most of the samples ranging from 0·6 to 1·3 p.p.m. Individual samples which had developed "oiliness" contained 1·5 p.p.m. or above, the average copper content being 2·3 p.p.m. The highest copper content met with was 4·0 p.p.m. in a sample which was objectionably "oily." The iron content of 10 pasteurised milk samples ranged from 1·7 to 3·8 p.p.m.

(*c*) *Sterilised milk.* Four samples of sterilised milk showed a copper content of from 0·4 to 1·2 p.p.m. The method of sterilisation in bottles does away with much of the possibility of metallic contamination during processing. Further, the oxygen-free nature of sterilised milk in airtight containers rules out the deteriorative effect of the presence of any abnormal amounts of copper on the milk fat. The range of iron content was from 1·6 to 2·8 p.p.m.

Detection of points of entry of copper into milk during processing.

The occurrence of "oiliness" in pasteurised or other milk calls for careful examination of the processing plant in order to detect the source of the contaminating metallic catalyst. In advisory work, such a problem was attacked by taking samples at various points in the processing and determining the copper content of the samples. When the offending piece of plant had been detected, one was in a position to advise as to its replacement or retinning to avoid further trouble. This scheme has been found to work well in practice. The following examples illustrate some results obtained:

Case I. Copper content of preliminary advisory sample = 2·3 p.p.m. Sampling at various points of the plant was carried out. The copper content of the samples at the cited points in parts per million were as follows: holder, 0·8; hot milk pump, 0·9; heat exchanger, 1·10; balancing tank, 1·10; filler, 1·10; sample kept for 10 minutes in the filling valve, 1·10.

The source of the highest copper contamination was the heat exchanger, the plates of which were found to expose large areas of bare copper surface to hot milk. When these plates were retinned the occurrence of "oiliness" in the product disappeared. In a similar investigation in another case, the cooler was shown to be the source of contamination, the tin having been abraded off the copper during cleaning operations.

Case II. Another example was afforded by a case of "oiliness" in Certified milk, where the contamination with copper occurred in the bottle-filling machine.

Copper content (in p.p.m.). Initial advisory samples, 3·28 and 3·33. Before passing through the bottler, 1·30; after bottling, 3·33. On cutting out the bottler, the trouble ceased.

On sampling the milk previous to bottling, the following contents of copper (in p.p.m.) were obtained:

Straight from the cow, 0·8; before passing over the cooler (after milking machine), 1·0; after passing over cooler (*a*) first runnings, 1·3; (*b*) full runnings, 1·1.

A small amount of contamination occurred from the cooler, the effect being most pronounced in the first runnings over the cooler. The high contamination with copper in the bottler was found not to be due to actual solution of copper by the milk but to the abrading effect of metal on a bronze base plate with which the milk was continually in contact.

B. *Cheese.*

The high biological oxygen demand in cheese owing to the concentrated micro-organic activity precludes any oxidative deterioration of the fat although abnormal amounts of copper might be present. A few copper determinations, have, nevertheless been made on cheese, including those on some

continental types. The following figures illustrate the range of copper content found (in p.p.m.).

(*a*) Cheese made at the National Institute for Research in Dairying, 0·6.

(*b*) New Zealand Cheddars, 2·1; 1·9; 1·0.

(*c*) Camembert, 6·0.

(*d*) Petit Gruyère, 9·2.

(*e*) Emmenthal, 18·1.

The types (*c*), (*d*) and (*e*) were made in copper vats which explained the abnormal copper content.

The iron content of cheese was found to vary from 6·0 to 16·0 p.p.m. Thus about 30 to 80 per cent. of the iron of the milk appeared in the cheese, most of the extraction occurring in the acid whey, especially in the press whey. Experiments in other directions have shown that with increasing acidity of the medium the "fixing" of heavy metal by milk protein is decreased.

C. *Dried milk and dried whey products.*

Two samples of dried milk contained 2·8 and 4·6 p.p.m. of copper, and 16·6 and 28·7 p.p.m. of iron. The copper contents were within the range expected from the contents in the fluid milk, but the iron contents are higher owing possibly to contamination during the drying on iron rollers. Two samples of dried separated milk and whey manufactured by the roller process with the use of a preheater and concentrator contained 18·2 and 12·6 p.p.m. of copper (iron not determined).

D. *Butter.*

Table I shows the iron and copper content of various samples of butter, most of which had developed taints due to fat oxidation.

The relatively high content of copper and iron in hand-made butter compared with the content of these metals in milk from which the butter was produced is explained by the fact that a heavy metal radicle concentrates itself at the fat-serum interface as a metal proteinate, with the result that the curd of the butter is relatively heavily laden with heavy metal. Factors governing the metallic content of butter are the curd content and the acidity of the cream before churning. The Dominion butters are churned from cream at low acidity (0·09 to 0·18 per cent. lactic acid) and are expected to contain the maximum possible amount of "native" iron and copper. It will be seen that these values are greatly increased in the faulty samples of butter, showing considerable contamination of the cream (or milk) with these metals before churning. Full-flavoured butter made from ripened cream would be expected to contain slightly less of the heavy metals. This has been borne out in experimental work.

Table I.

Source and number of sample	Copper p.p.m.	Iron p.p.m.	Acidity ml. normal alkali required per 100 g.	Remarks
N.Z. 1	9·30	—	—	Fishy, quickly followed by tallowiness (whey butter)
N.Z. 2	1·40	—	1·77	No taint
N.Z. 3	2·00	—	9·10	Slightly fishy
N.Z. 4	1·20	—	—	No taint
A. 1	1·60	—	3·08	No taint
A. 2	2·00	6·60	0·93	Fishy
A. 3	0·90	5·50	1·42	Fishy
A. 4	2·20	2·50	1·14	Fishy
A. 5	1·70	3·80	1·32	No taint
A. 6	3·20	1·70	3·45	Fishy
A. 7	2·30	1·30	2·48	Fishy
A. 8	Trace	3·00	7·90	Acid and off flavour
A. 9	Trace	2·40	8·00	
A. 10	1·30	1·90	—	Good samples of Australian butter
A. 11	1·80	1·80	—	
A. 12	1·30	1·10	—	
A. 13	1·64	2·30	—	
A. 14	1·60	1·60	—	
A. 15	3·60	2·20	—	Fishy
A. 16	2·00	0·90	—	Slightly fishy
A. 17	2·40	2·40	—	Fishy
Ukranian	3·70	3·20	1·80	Fishy
Siberian	Trace	2·90	1·49	Rank flavour
Mid-Wales	Trace	1·75	3·30	Old sample—fishy
N.I.R.D.	0·50; 0·88 0·56; 0·76	1·12; 0·96 0·86; 1·31	—	Fresh, wholesome

(N.Z. = New Zealand; A. = Australia.)

A QUICK METHOD OF DETECTING THE CONTAMINATION OF MILK WITH BLOOD CELLS.

The determination of iron in the centrifugate of milk suspected of containing blood cells can be carried out in about 15 minutes, and is direct evidence of the presence of iron in a centrifugable form. (Iron as proteinate by direct contamination with iron solution would not be separable by centrifuging and solid iron compounds could be filtered off.)

Thus 75 ml. of suspected milk were centrifuged and as much as possible of the supernatant layer poured off. The remaining 5 ml. were ashed and found to contain 13·1 p.p.m. of iron. Allowing 2 p.p.m. as the original content of iron in the milk, it was found that 0·056 mg. of iron had been added, corresponding to 14 mg. of haemoglobin (which contains 0·4 per cent. iron) or 0·019 per cent. of the milk or 0·13 per cent. of blood in the milk.

SUMMARY.

The range of the copper and iron content of 80 samples of fresh, pasteurised and sterilised milk are given. Examples of investigations on the point of entry of copper into milk during processing are given. As a minimum limit, 1·5 p.p.m. of copper in milk can be expected to produce "oiliness" in milk.

The copper content of dried milk is usually normal but the iron content is increased above that expected from the iron content of fluid milk.

Cheese made in copper vessels has a high copper content; and the iron content of cheese is variable.

Butter contains on an average about 0·5 p.p.m. of copper and 1 p.p.m. of iron. Factory made butter invariably contains more iron and copper.

The more a milk product is processed, the greater is the contamination with heavy metal.

An example of determining the amount of blood in milk by a determination of the iron content is given.

REFERENCES.

(1) Mattill (1927). *J. Amer. Med. Assoc.* **89**, 1505.
(2) Hess and Unger (1921). *Proc. Soc. Exp. Biol. Med.* **19**, 119.
(3) Lampitt, *et al.* (1926). *Analyst*, **51**, 327.
(4) Elvehjem and Lindow (1929). *J. Biol. Chem.* **81**, 435.
(5) Supplee, *et al.* (1930). *J. Nutrition*, **2**, 451.
(6) Elvehjem, *et al.* (1929). *J. Biol. Chem.* **83**, 27.

(*MS. received for publication June* 23, 1931.)

31. THE DETERMINATION OF COPPER AND IRON IN DAIRY PRODUCTS

By W. WILLIAMS.

New Zealand Co-operative Dairy Co. Ltd., Research Laboratory, Hamilton, New Zealand.

The importance of the effect of metallic contamination of dairy products, has been stressed by a number of different authorities. Hunziker (1) has carried out extensive experimental work on the metallic contamination of butter and cream. His work shows that the presence of traces of copper and iron have the power to accelerate bacterial action in butter and also to stimulate oxidation and decomposition of its ingredients. The action of metallic salts is further augmented by producing intense progressive flavour, deterioration in the dairy product with age, due to oxidation and catalytic changes, metallic, tallowy, and fishy flavours being produced.

Davies (2) states that the development of oxidative rancidity and other effects of auto-oxidation of fat are accelerated enormously by the presence of minute traces of metallic salts especially those of copper and iron, as little as one part of these metals per million of fat shortens the induction period of oxygen up-take. Supplee and Bellis (3) have shown that the average copper content of freshly drawn cows' milk is 0·52 parts per million (average of 23 samples). Rice and Miscall (4) state that when milk contaminated with copper is passed through a separator, the metal distributes itself between the cream and skim milk fractions approximately in proportion to the water. It would seem, therefore, that it is dissolved in the water. The results show conclusively that the copper does not go with the fat, and is therefore not dissolved in the fat.

Although a large amount of experimental work has been reported in literature by various workers on the effect of metals on the keeping quality of butter, most of the work has been carried out by the addition to cream or butter of definite amounts of copper and iron salts. Very few results have been published showing the actual amount of metallic contamination which takes place, during the manufacture of butter on an extensive scale, as carried out in New Zealand; and methods of determining copper and iron in butter have not been fully described.

The following is a brief outline of equipment and the process of manufacturing butter, as used in the factories concerned in these experiments and which is representative of general New Zealand practice. The cream after weighing and sampling is run into tinned copper vats of several hundred gallons capacity, where it is tested for acidity and the requisite amount of

sodium bicarbonate solution is added and mixed in to reduce the acidity of the pasteurised cream to a value of 0·08 to 0·10 per cent. lactic acid. The cream is then pumped through sanitary piping to a regenerator where it absorbs heat from the hot cream on its return to the coolers and then through two flash pasteurisers working in series. In the first pasteuriser the temperature is raised to above 150° F., and in the second to 180° F.–200° F. The hot cream then passes over a series of surface coolers and finally over a direct expansion cooler and is pumped through sanitary piping into large holding vats usually of tinned copper. It is held at a temperature of 40° F.–50° F. overnight and churned next day.

Owing to New Zealand butter having to be kept in storage for a great length of time before it reaches the consumer in England, the presence of metallic contamination presents a serious problem and special precautions must be taken to avoid any contamination at any stage either on the farm or during manufacture.

Contamination on the farm has its source in rusty cans and other utensils and in poorly tinned milking machine plant and separators. In many districts it is also brought about by the water supply containing a high amount of iron. Contamination in the factory is possible from a number of sources, and the elimination of trouble is often a difficult matter especially where exposed copper is showing.

The determination of very small quantities of copper and iron presents three problems as follows:

(*a*) The selection of a colorimetric method of sufficient sensitiveness.

(*b*) The elimination from the blank of practically all copper and iron so that the colour given in the blank will be only very slight compared to the sample.

(*c*) The devising of a method of ashing whereby large quantities of such products as butter and cream can be conveniently handled.

A. The selection of the colorimetric method was made on the results of a considerable amount of work on the various methods described in the literature. The most suitable method for iron was found to be the modification by Smith and Cook (5) and Stokes and Cain (6) of the thiocyanate method, *i.e.* the ferric thiocyanate is extracted from the solution in a small volume of amyl-alcohol-ether mixture, and comparison is made with a standard in the same solution.

For the estimation of copper, the dimethyl-glyoxime method of the Research Department, Woolwich (7), did not give satisfactory results. The greater portion of the work on copper estimation herein described was carried out by the xanthate method (3). The sodium diethyl-dithio-carbamate method of Callan and Henderson (8), however, proved more sensitive than the xanthate method, and has been used in later work.

B. It has been found impossible to eliminate completely the whole of the contamination, which is always found in the blank determinations which are

run side by side with the actual sample. This has made it very important that in the determination of both copper and iron by colorimetric methods both metals must be in sufficient quantities to give considerably greater colour than the blank. By the usual ashing methods where only about 10 g. of butter is taken for the determination, it was found to be a difficult matter to obtain good duplicate and triplicate results owing to the high blanks obtained from crucibles and apparatus even when porcelain burners were in use. The slight increase in colour in the final matchings of the samples and blanks and the slight variations in the blanks themselves caused, therefore, variations in the duplicate and triplicate results. The elimination of practically all the metals from the blanks has been accomplished by very careful study of the source of the contamination and the elimination of each source in turn. The use of pyrex beakers and the wet ashing process has been a big factor in this connection.

C. The difficulty of handling sufficient quantities of butter and cream has been overcome by the wet ash process and this method has been adopted in most of the work that has been carried out. The following method was finally adopted:

50 g. of butter or 25 g. of cream are weighed into a 150 c.c. pyrex beaker. Nitric acid (15 c.c.) is added and the mixture is boiled on a hot plate for 5 minutes, with constant stirring to insure the thorough extraction of copper and iron from the fat and to prevent bumping, which usually takes place due to the large amount of fat present.

Extraction of fat. The sample is cooled, but the temperature is not reduced below the melting point of the butterfat, benzolene (100 c.c.) is added and thoroughly stirred until all of the fat is dissolved. The benzolene extract is poured off into a 500 c.c. flask, care being taken that none of the acid portion is removed.

Two further extractions are made with 100 c.c. benzolene. The benzolene extract is thoroughly shaken in the flask with three successive quantities of 15–25 c.c. of hot water for extraction of any acid solution. Each quantity when it has settled is transferred by means of a pipette to the beaker containing the main portion of nitric acid solution (the extraction and washing may, with equal convenience, be carried out in a separating funnel).

Digestion. The acid extract is concentrated to a volume of about 25 c.c., and concentrated sulphuric acid (25 c.c.) is added (sulphuric acid A.R. has been found to be almost free from iron and copper). The mixture is strongly heated on a hot plate for half an hour to one hour when partial digestion will have taken place, sodium nitrate (pure) is carefully added, a few crystals at a time to avoid violent action and possible spurting taking place. The addition is continued until the solution becomes quite clear. Usually about 2 or 3 g. is sufficient, but if the solution does not clear, it is best to heat again for 10 to 20 minutes and add a further quantity of sodium nitrate. Butter clears with less sodium nitrate than cream.

D. The solution is now strongly heated until the whole of the sulphuric acid is driven off. When fuming ceases the sample is cooled and with complete digestion a white crystalline mass is obtained. This mass is dissolved by boiling with 10 c.c. of diluted hydrochloric acid (5 parts acid to 2 parts water), then about 25 c.c. of water is added and the solution boiled for about 5 to 10 minutes, cooled and made up to 50 c.c. volume.

Iron. 10 c.c. of solution are placed by means of a pipette in an 8 in. × 1 in. glass tube. The tubes must be of the same diameter and of a clear white glass. The author used special clear glass tubes with glass stoppers. 10 c.c. of water and 5 c.c. of ammonium thiocyanate solution are added, and the mixture shaken thoroughly. 10 c.c. of a mixture of amyl alcohol and ethyl ether are then added, the tube is corked and shaken thoroughly. The red ferric thiocyanate colour extracted by the ether mixture is compared against standards containing known amounts of iron. Standards must contain a few drops of HCl or the ferric thiocyanate extract will be cloudy.

Copper. Xanthate method. The remaining 40 c.c. of solution is returned to the beaker, the solution is made ammoniacal, filtered and washed three or four times with water, the precipitate is dissolved in dilute hydrochloric acid and precipitated with ammonia, filtered and washed into first filtrate The solution is now boiled until ammonia is completely driven off, and allowed to cool. One drop hydrochloric acid, then 2·5 c.c. 0·5 *N* sodium hydroxide and 8 c.c. of 0·1 *N* hydrochloric acid are added. The solution should be neutral but on no account alkaline. If the sample is suspected of having a large amount of copper present, such as may be the case with certain cream, it is advisable to make up to a definite volume at this stage and take an aliquot so that varying amounts may be compared. For butter it is best not to make up but wash the whole of the solution into a 100 c.c. Nessler tube and add 10 c.c. of xanthate reagent, and to match colour produced, after standing about 10 minutes, against standards containing known amounts of copper. Blanks containing all reagents must be determined with sample and deducted.

E. *Sodium diethyl-dithio-carbamate method.* The solution remaining after removal of the aliquot portion for iron determination is returned to the original beaker. Ammonia is added until the action is alkaline, and the solution is filtered direct into a Nessler tube. The precipitate is redissolved in hydrochloric acid solution, again made alkaline with ammonia and filtered into a Nessler tube. The precipitate is washed a few times with water. The solution is made up to 100 c.c. and an aliquot portion is taken (or the whole may be used if necessary). Carbamate solution (10 c.c.) is added, the mixture stirred with a glass rod, made up to 100 c.c. with water, and again thoroughly stirred. The colour is matched against standards containing known amounts of copper.

In the presence of very minute amounts of copper, the colour produced by the carbamate may be extracted by definite amounts of chloroform and matched against standards treated in the same way. The above method is not only more sensitive than the xanthate method, but is also more rapid. It requires a very

limited range of glassware and chemicals, and, therefore, is much less liable to error due to possible contamination from these sources.

Throughout this work no difficulty has been experienced in the method for iron estimation through interference due to phosphates as claimed by Elvehjem and Hart (9) and Walker (10). This is in agreement with the findings of Leeper (11) who showed that the incidence of phosphate interference has been exaggerated. The latter worker shows that pyrophosphates do cause trouble but that orthophosphates have no effect, even when present in relatively large amounts, viz. up to 100 mg. With the wet ashing method as outlined above the formation of pyrophosphate does not occur. One of the inherent difficulties in the determination of very small quantities of iron in foodstuffs is therefore obviated.

SOLUTIONS REQUIRED AND PREPARATION OF GLASSWARE.

Glassware. All glassware used has been pyrex and this has been used for nothing else but the copper and iron determinations. It was first thoroughly washed with hot nitric and hydrochloric acids and then with glass distilled water.

Solutions. (1) Nitric acid redistilled through glass condenser.

(2) Sulphuric acid (chemically pure). This may be redistilled in pyrex glassware, if necessary.

(3) Hydrochloric acid, redistilled through glass condenser into (4).

(4) Water redistilled through glass condenser.

(5) Sodium nitrate (pure crystals). This may be recrystallised with (4) in glass vessels.

(6) 5 per cent. solution ammonium thiocyanate made up with (4). A portion of this solution is extracted with the amyl alcohol ether mixture for the detection of any iron present. If present, the whole of the solution may be extracted with a portion of the ether-alcohol mixture.

(7) Amyl alcohol redistilled through a glass condenser.

(8) Ethyl ether redistilled through a glass condenser.

(9) Ammonia redistilled through a glass condenser into (4).

(10) *Standard iron solution.* Dissolve 0·7022 g. of ferrous ammonium sulphate in 100 c.c. water, add 5 c.c. sulphuric acid. Warm and titrate with potassium permanganate solution until just pink. Cool and make up to 1 litre. (1 c.c. = 0·1 mg. iron.)

(11) *Standard copper sulphate.* (*a*) 3·9283 g. copper sulphate made up to 1000 c.c. with (4). (1 c.c. = 0·001 g. copper.) (*b*) 10 c.c. of (*a*) made up to 100 c.c. with (4). (1 c.c. = 0·00001 g. copper.)

(12) *Benzolene.* Redistilled through glass condenser.

(13) *Sodium diethyl-dithio-carbamate.* 0·1 per cent. solution in (4).

(14) *Filter papers.* A packet of No. 41 9 cm. Whatman papers is extracted in a glass vessel for about 48 hours with dilute "glass distilled" hydrochloric acid. The acid is then poured off and the papers are washed several times with "glass distilled" water.

Results obtained.

These results will not be discussed in detail but some of the more interesting points will be indicated. Whole milk, skim milk, buttermilk, cream and butter samples have been analysed but the work has been mostly concerned with the contamination of cream and butter by copper and iron in the factory.

(1) The following representative results have been obtained with different grades of cream received at various factories. The grading of cream is carried out by qualified cream graders, on receipt of the cream at the factory, and classification is made according to aroma and flavour as superfine (highest grade), 1st and 2nd grade. Whey cream is kept separate from the other grades. The results are given in duplicate.

Sample	Grade	Copper p.p.m.	Iron p.p.m.
(A)	1st	1·4, 1·6	2·0, 1·6
(B)	1st	1·4, 1·6	1·5, 1·1
(C)	1st	2·2, 2·6	2·0, 2·0
(D)	Superfine	1·2, 1·2	1·2, 1·2
(E)	Whey cream	0·95, 0·70	7·0, 7·0
(F)	,,	1·7, 1·7	7·8, 7·0

These results indicate the increasing amounts of metallic contamination in the 1st grade and whey cream above that of the superfine cream.

(2) The following representative results have been obtained with different grades of butter manufactured at various factories.

		Copper p.p.m.	Iron p.p.m.
(A)*	Butter made from 1st grade cream	0·2, 0·2	0·5, 0·6
(B)*	Butter made from 2nd grade cream	0·5, 0·5	1·1, 0·9
(C)	Superfine butter (6 different samples)	0·2, 0·2, 0·2, 0·25, 0·2, 0·25	0·8, 0·5, 0·6, 1·0, 0·5, 0·5
(D)	2nd grade butter (3 different samples)	0·2, 0·2, 0·25	1·7, 1·7, 1·2
(E)	Whey butter (2 different samples)	0·6, 0·6	2·5, 2·5

* In (A) and (B) duplicate results on the same samples are given.

These results show the normal content of superfine butter to be 0·2 or 0·25 p.p.m. of copper and 0·5 to 1·0 p.p.m. of iron, but with 2nd grade butter, although in these cases the copper is normal the iron has increased to 1·2—1·7 p.p.m. In whey butter both copper and iron have greatly increased.

(3) Results showing contamination of first cream coming over the pasteurising and cooling plant at different butter factories (A, B, C, D).

		Duplicate results	
		Copper p.p.m.	Iron p.p.m.
(A)	(1) First few gallons through plant	1·65, 1·90	1·2, 1·2
	(2) 5 minutes after starting	1·30, 1·15	0·8, 0·4
	(3) 20 minutes after starting	0·50, 0·30	0·6, 0·6
	(4) Full vat	0·90, 0·90	0·8, 0·8
(B)	(1) First few gallons through plant	8·00, 8·00	1·8, 1·8
	(2) 5 minutes after starting	4·00, 4·00	1·4, 1·4
	(3) 20 minutes after starting	0·75, 0·75	1·6, 1·6
	(4) Full vat	2·25, 2·25	1·6, 1·6
(C)	(1) First few gallons through plant	4·00, 4·00	2·0, 1·8
	(2) 5 minutes after starting	3·50, 3·75	1·6, 1·6
	(3) 20 minutes after starting	0·75, 0·75	1·2, 1·2
	(4) Full vat	0·75, 0·75	1·2, 1·2
(D)	(1) First few gallons through plant	4·85, 4·85	1·8, 2·2
	(2) 5 minutes after starting	4·85, 4·85	1·4, 1·2
	(3) 20 minutes after starting	1·10, 1·35	1·0, 1·0
	(4) Full vat	0·85, 0·85	1·2, 1·2

The cream was being pumped through the system at a rate of approximately 800 gallons per hour and the full vat represented 700 to 800 gallons of cream.

(4) Results showing distribution in cream, butter and buttermilk in a factory having metallic trouble.

	Copper p.p.m.	Iron p.p.m.
(A) Whey cream before pasteurising	0·8	7·2
(B) Whey cream after pasteurising and cooling	4·5	8·2
(C) Whey cream after 4 hours in vat	5·6	8·2
(D) Whey cream after 16 hours in vat	8·1	8·5
(E) Butter-milk from above cream (D)	9·1	11·0
(F) Butter from above cream (D)	2·1	7·2
(G) Butter from above cream (D)	2·1	7·7

In this case pasteurisation was being carried out in a vat with coils for heating and cooling. Excessive contamination was taking place.

(5) Results on samples taken at different points from plant.

	Copper p.p.m.
(A) Cream at neutralising vat	0·5
(B) Cream at top of pasteuriser	0·9
(C) Cream at top of cooler	1·3
(D) Cream at top of expansion cooler	1·4
(E) Cream in vat next day as ran to churn	1·6
(F) Buttermilk from above cream	2·0

The plant in this case was showing a good deal of exposed copper especially in the flash pasteurisers and the gradually increasing amount of contamination is evident.

SUMMARY.

1. Two colorimetric methods for determination of copper have been described, viz. the xanthate and the sodium diethyl-dithio-carbamate. Both methods have given satisfactory results but the carbamate method has proved to be more sensitive and less liable to error from possible contamination than the xanthate method.

2. The colorimetric determination of iron by the modification of the ferric thiocyanate method has been proved to give a very high degree of accuracy and excellent results have been obtained.

3. A method of wet ashing has been described whereby organic matter is oxidised by the use of concentrated sulphuric acid and sodium nitrate and copper and iron extracted from the fat.

4. A number of results are given showing the amount of copper and iron contamination that the cream is subjected to during the course of butter manufacture, and the distribution of copper and iron in the cream, butter and buttermilk.

REFERENCES.

(1) Hunziker (1927). *The Butter Industry*, p. 209.
(2) Davies (1928). *J. Soc. Chem. Ind.* June 29, 185–7 T.
(3) Supplee and Bellis (1922). *J. Dairy Sci.* **5**, 455.
(4) Rice and Miscall (1923). *J. Dairy Sci.* **6**, 261.
(5) Smith and Cooke (1926). *Analyst*, **51**, 503.
(6) Stokes and Cain (1907). *J. Amer. Chem. Soc.* **29**, 409.
(7) Research Dept. Woolwich (1929). *Analyst*, **54**, 333.
(8) Callan and Henderson (1929). *Ibid.* **54**, 650.
(9) Elvehjem and Hart (1926). *J. Biol. Chem.* **67**, 43.
(10) Walker (1925). *Analyst*, **50**, 279.
(11) Leeper (1930). *Ibid.* **55**, 59.

(*MS. received for publication June* 19, 1931.)

32. A CONTROLLED CHEESE-RIPENING ROOM

By N. S. GOLDING, M.Sc. (Iowa), B.S.A. (Tor), N.D.A., N.D.D.
University of British Columbia.

(With 1 Figure.)

Introduction.

If research work in cheese ripening is to be conducted on an entirely satisfactory basis, a complete control of both temperature and humidity of the ripening room must be available.

Though temperatures are relatively easily controlled, the factor of shrinkage or loss of moisture from the cheese is a more difficult problem. Further, should such loss of moisture be an additional and variable factor due to differences in humidity of the ripening room, research work in cheese ripening is seriously handicapped. It is realised that different cheese, whether large or small, high or low in moisture, new or mature, lose moisture at different rates even though kept under exactly the same conditions of storage. Also, the identical cheese cease to remain the same due to various rates of ripening, if studied as to their loss of moisture at different temperatures.

For these reasons we can never hope to express the control of humidity in terms of loss of moisture from a standard cheese. Further, the percentage relative humidity is not a suitable measure when temperatures of ripening are to be varied as the water-holding capacity of the air declines with temperature. Probably the best control of evaporation could be said to be attained were the conditions such that the loss of water from a definite exposed surface of water was at a constant rate at whatever ripening-room temperature selected. Such a control after being once properly set should be entirely automatic whatever the conditions outside the room. Until such a cheese-ripening room accomplishes this ideal, the work on a constant temperature and constant humidity ripening room can be considered, as at present, only in progress.

Principles for temperature control.

Temperature control of rooms, incubators and water baths is accomplished by thermostats which are based on the difference in rate of the coefficient of expansion of various substances. If a temperature is required in the room always above that of the outside air only one thermostat is required which controls the amount of heat going to the room and so keeps it at constant temperature. When, however, the temperature of the room is sometimes above and sometimes below that of the outside air, two thermostats are required, one controlling the supply of heat when the air outside is colder than the room, and the other controlling removal of heat when the air outside is warmer than that of the room. The last case usually applies to cheese-ripening rooms.

Principles for humidity control.

Two principles for controlling humidity in a room or incubator are available and suitable for consideration.

The first is that given by Spencer (2) which states: "A saturated aqueous solution in contact with an excess of a definite solid phase at a given temperature will maintain a constant humidity within any enclosed space around it. By properly selecting the salt to be used almost any desired degree of humidity can be secured and controlled in this way. A number of salts suitable for this purpose are displayed in the accompanying chart and tables together with the percentage humidity prevailing above their saturated solutions at different temperatures." This principle is highly suitable to apply in small incubators where only relatively small quantities of water have to be given up or taken from the air.

However, in the ripening of cheese, particularly during the early stages, considerable loss of water makes the principle unsuitable to apply. The Kingston cheese for which the ripening room was constructed, lost moisture at the rate of about 5 per cent. for the first 6 to 8 days of ripening. Therefore, it is definite that a principle of obtaining constant humidity must be used that will take care of a very considerable quantity of water if many cheese are to be ripened in the same room.

With these facts in mind the principle was adopted that when saturated air at a given temperature is raised a certain number of degrees a definite humidity is obtained. The required humidity is obtained by adjusting the thermostats in the cool box and in the ripening room respectively to certain temperatures according to the figure given by Brabbée (1).

The cheese-ripening room.

The ripening room is 11 ft. 1½ in. long, 9 ft. wide and 7 ft. high, the whole being constructed in sections. This room is well insulated, being constructed as follows: 1 in. flooring, 2 in. pressed cork, 1 layer of building paper, 2 in. pressed cork, 1 in. flooring.

The usual type of cold storage door is fitted on one side. A 1 ft. slide ventilator is fitted in centre of the room to be used when required.

Cooling surface.

The cooling surface (Fig. 1) is a narrow tank 5 ft. long, 3 ft. 4 in. high and 3 in. wide which is supported from one of the side walls. A drip tray is fitted below the tank to recover the condensed water which drains to outside the room. The whole is boxed in with 1 in. flooring. The warm air enters into the box at the four controlled inlets at the top. These inlets can be extended into the middle of the room by galvanised pipes. One or more of these inlets are used as required. The air entering at one or more inlets to the box falls past the cooling surface and the excess moisture is condensed on the cold tank, dew-

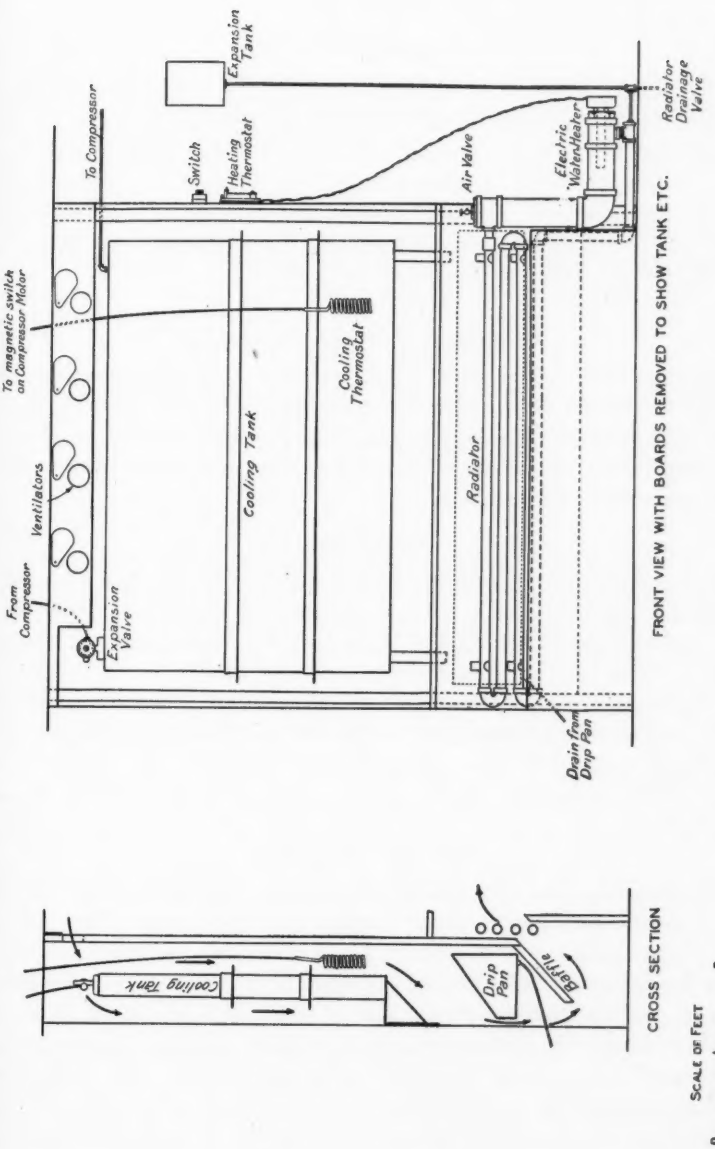

Expansion Tank
To Compressor
Switch
Heating Thermostat
Air Valve
Electric Waterheater
Radiator Drainage Valve
To magnetic switch on Compressor Motor
Ventilators
Cooling Tank
Cooling Thermostat
Radiator
From Compressor
Expansion Valve
Drain from Drip Pan
FRONT VIEW WITH BOARDS REMOVED TO SHOW TANK ETC.
Cooling Tank
Drip Pan
Baffle
CROSS SECTION
SCALE OF FEET
0
1
2

Fig. 1.

point being reached as the air gets to the bottom of the box. The air then flows past the baffle and then to the radiator of 1 in. pipes, and is raised to the required temperature of the ripening room.

Controls.

Temperature control of the cooling box (Fig. 1) is as follows: A thermostat, connected to a Penn Magnet switch, type *L*, range 25 to 80° F., is fitted in the cooling box. The switch is connected to the electric motor which operates the half ton Universal Coolers compressor situated on the top of the ripening room. Thus the compressor which cools the tank by means of expansion coils operates only when the cooling box is above the required temperature and automatically shuts off when this temperature is reached.

Temperature of the ripening room.

The temperature of the cheese-ripening room is controlled by an ordinary bar type thermostat fitted with heavy platinum points which connects up to a 600 watt hot water heater as shown in Fig. 1. This thermostat is so adjusted that when the ripening room reaches the required temperature the current which warms the coils is shut off.

Humidity.

The humidity control consists in adjusting the two thermostats so that the saturated cold air is raised in temperature a certain number of degrees as it passes the warm coils, therefore giving a definite relative humidity. The required humidity can be obtained by consulting the figure given by Brabbée(1) and adjusting the thermostats to the required temperatures. However, if this figure is not available, first adjust the thermostat in the ripening room to the temperature required for ripening the cheese and then alter the thermostat in the box until the desired humidity is shown on the hygrometer in the cheese-ripening room.

Application in practice.

The cheese-ripening room just described has worked satisfactorily for the past 18 months for Kingston cheese ripening at a temperature of 60 to 65° F. and a relative humidity of 88 per cent. The chief difficulty experienced is when only a few cheese are being ripened and there is not sufficient moisture given off to keep up the humidity even if most of the inlets to the box are shut. A small humidifier in the ripening room would obviate this difficulty. In addition to the humidifier an automatic control on the intake of air to the cooling box would be an advantage over the hand controls now in use. The proposed plan is to have a receptacle containing a saturated solution of a salt which gives up water at the required humidity(2). The reduction in weight of this solution due to loss of water would operate to close the inlets to the box and so decrease the circulation of air in the ripening room. On the other hand, excessive humidity

would be counteracted by the solution taking up water increasing in weight and opening the inlets to the box and thus increasing the circulation of air in the ripening room.

REMARKS.

It cannot be said that the cheese-ripening room has reached the ideal we hope to attain, but the results obtained are unquestionably a great improvement on any we have had in the past.

ACKNOWLEDGMENTS.

The construction of the ripening room was made possible by the Research Fund established jointly by the Empire Marketing Board and the University of British Columbia.

REFERENCES.

(1) BRABBÉE, C. W. (1927). *Heating and Ventilation.* 1st ed. Fig. 188. McGraw-Hill Co. New York.

(2) *International Critical Tables* (1926). 1, 67. McGraw-Hill Co., New York.

(*MS. received for publication, July* 9, 1931.)

33. A CONTRIBUTION TO THE BACTERIOLOGY OF COMMERCIAL STERILISED MILK

PART I. GENERAL

By E. R. HISCOX, M.Sc. and M. I. CHRISTIAN, Ph.D.

The National Institute for Research in Dairying, University of Reading.

(With 1 Chart.)

In spite of the fact that commercial "sterilised" milk is strongly heated during the process of manufacture, it is rarely *sterile* in the full sense of the word. The commercial process of sterilisation may, in fact, act as a means of selecting certain types of heat-resistant bacteria and of providing them with conditions more favourable for growth.

The details of the process differ slightly in various factories, but the heat applied to the milk is invariably sufficient to ensure that only heat-resistant bacterial spores will survive. The method in most general use in England is the heating of the bottles of milk in large steam chests at 210–212° F. for 30 minutes. At the end of this time the bottles are immediately sealed (if swing-stopper bottles are used) and allowed to cool, either in the air or in water. In some instances the time of heating is prolonged, *e.g.* to 90 minutes, in others the temperature is raised, *e.g.* to 220° F., by the use of steam under pressure.

A detailed account of the processes in use in France, and of the resulting chemical changes, has been given by Pellet(1). On the whole the heating is more severe, *i.e.* is carried out at higher temperatures and for longer periods than is the general practice in England. The less drastic the heating process, the less marked will be the "cooked" flavour of the milk, but the greater is the danger of the survival of spore-bearing bacteria.

The bacterial flora of commercial sterilised milk will obviously be of a special type, and if the bottles are satisfactorily sealed will be limited to spore-bearing bacteria highly resistant to heat. Most of these are able to develop in the milk, and may bring about marked changes with resultant spoilage and loss to the industry.

It appears that the bacteriology of commercial sterilised milk has received little attention, and scientific literature on this subject is very scanty. No British contribution is available, and reference must be made to the German works published towards the end of the last century. Flügge(2) gave details of the various methods of preparation in use at that time, and emphasised the fact that the sterilisation of milk on commercial lines would, at most, only reduce the number of bacteria. The results of his investigations of the different types of spore-bearing bacteria which survived the heating process and were commonly found in commercial sterilised milk showed that they were chiefly

of the peptonising type. This work was confirmed by Weber (3), Hueppe (4) and others, who considered this question in relation to outbreaks of intestinal disorders of infants fed on commercial sterilised milk. Later Huss (5) isolated and described an organism which was responsible for clotting and the development of a bitter taste in tins of sterilised milk. It differed essentially from the types described previously, and brought about only a comparatively slight digestion of the casein. No record has been traced of organisms similar to the taint-producing, non-clotting, non-peptonising type (to be described later in this paper), which, as present-day experience shows, is of major importance in the spoilage of English commercial sterilised milk.

Routine examination of commercial sterilised milk.

This paper deals with the results of a prolonged study of English commercial sterilised milk, and gives a brief review of the chief forms of spoilage and the organisms associated with these changes. The work was begun in July, 1925, when bacteriological examinations were made of bottles of sterilised milk selected at random from the stock of a factory where milk was subjected to a temperature of 212° F. for 30 minutes. Two bottles were used for each test. Of these, one was tested on its arrival at the laboratory (*i.e.* the day after sterilisation), the other after storage. At first the second bottle was held at 37° C. until the milk showed definite signs of coagulation or digestion. Later, (September–November, 1925), it was stored at 22° C. and tested on the 7th day after sterilisation whether the milk showed visible change or not. The tests were made by plating on nutrient agar and by inoculation into litmus milk. The results indicated:

(1) That the majority of the samples had an appreciable bacterial content even on the day after sterilisation. With the advent of colder weather, the amount of initial infection decreased, and samples frequently appeared to be sterile on the day after sterilisation. But the corresponding samples stored at 22° C. almost invariably showed definite infection when tested on the 7th day, indicating the possibility of an initial infection in all samples, but of so slight a nature that it was not always detected by the methods employed.

(2) The reactions in litmus milk were of two main types:

(i) Coagulation or digestion of the casein with acid, alkaline or neutral reaction.

(ii) Rapid bleaching with the development of acidity, which was, however, not sufficient to coagulate the milk except on boiling.

At first, bacteria responsible for both types of reaction could be detected in most of the bottles, but later, from the middle of September onwards, the bacteria causing the latter reaction became dominant to the almost entire exclusion of the others. This may have been due to a change in the type of the initial infection of the milk, or, on the other hand, it may have been the result of the alteration of the temperature and period of incubation.

Evidence goes to show that it is only during the summer months that the

sterilised milk industry suffers severe loss as a result of bacterial spoilage of the milk, but even up to the end of November very few of the samples tested in the laboratory were really sterile. It is true that towards the end of this period the amount of initial infection had decreased to such an extent that the majority of the samples appeared to be sterile when tested on the day after sterilisation, but even so, with few exceptions, the duplicate bottles which were tested after storage at 22° C. for 7 days showed quite appreciable infection.

The results of the preliminary examinations thus raised several important points, but the number of tests (on 66 duplicate samples, examined over the period July–November) was too small to allow of generalisations on a product which showed such variable infection.

It was therefore decided to carry out a series of routine examinations in order to study the bacteriological content of samples of commercial sterilised milk for a period extending over at least 1 year. The tests were carried out weekly, on three dozen bottles which had, as before, been selected at random from the ordinary stock of a factory and dispatched on the day of sterilisation. Immediately on their arrival at the laboratory on the following day they were placed, in batches of 12, in:

(i) An incubator maintained at a constant temperature of 22° C. (72° F.) (approximately summer temperature).

(ii) A well-insulated cupboard (room temperature). This temperature varied with the season. The chart of a recording thermometer placed near the bottles showed no great fluctuations in temperature during the day, although there might be a marked rise or fall of the average during the week.

(iii) A cellar. No cold store was available at the time, but it was hoped that the cellar might provide a constant temperature lower than that of (ii). It proved, however, an unsatisfactory substitute for cold store, as the temperature varied with the season and at times was only slightly below that of (ii). The results of the tests at cellar temperature are therefore very similar to those at room temperature and may be regarded as confirmatory of these findings.

On the 5th, 6th and 7th days after sterilisation, four bottles were taken from each of the three batches and examined for sweetness and sterility. The results have been plotted on Chart 1 on which the unbroken lines represent the number of unsound (*i.e.* tainted or clotted), and the broken lines the number of unsterile samples at each weekly test. No definite increase in the number of unsterile samples could be observed from the 5th to the 7th day, indicating that it is possible to detect incipient spoilage on the 5th day. Later experience with cultures of organisms isolated during these examinations supported this assumption, for the characteristic reactions in milk culture could almost invariably be detected before the 5th day, especially when incubated at 22° C. For the purpose of this chart, therefore, the sum of the results of the tests on the 5th, 6th and 7th days has been taken to represent the complete result of the week's test.

Seasonal variations.

Certain facts become evident from a study of this chart.

(1) At no time of the year could absolute reliance be placed on the sterility of the product, although the infecting organisms were not necessarily numerous or active enough to bring about obvious changes in the milk during the period of storage. The period of least infection was during the early spring months (February to May inclusive). The data for sterility are not complete for the warm months of May to October, but since these months show a marked increase in the number of unsound samples it may be assumed that they also represent the period of greatest infection.

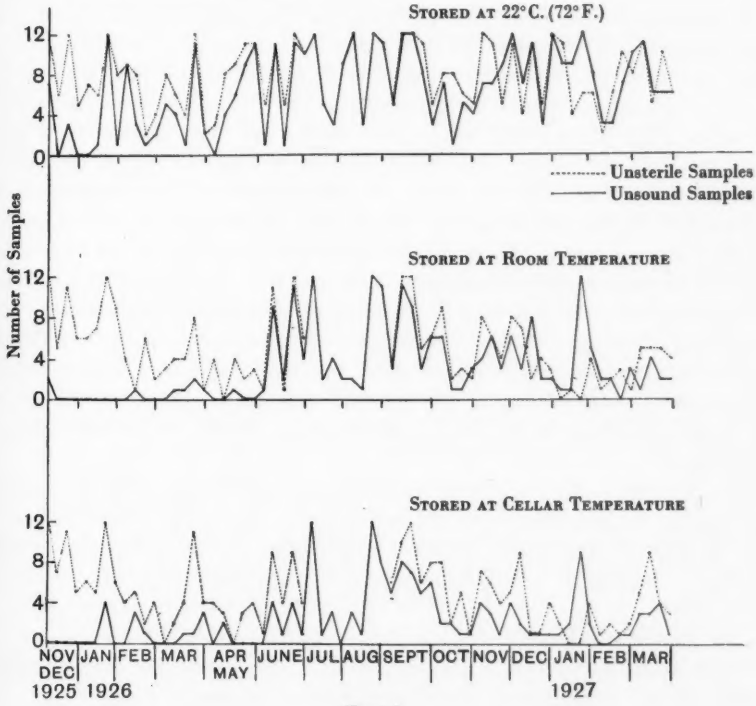


Chart 1.

The tests for sterility in this routine work could not be exhaustive or very delicate. As a rule they consisted of the addition of 1 c.c. of the milk to 10 c.c. of sterile litmus milk, and the recording of the results of incubation at 37° C., although sometimes quantities of 1 c.c. were also plated on nutrient or casein agar. A slight infection, therefore, might easily have remained unrecorded. This may explain why frequently the proportion of unsterile samples appeared to be greater for the batches stored at 22° C. than for the batches stored at the lower temperatures, since the more rapid multiplication of the bacteria at the

higher temperature would allow of a more ready detection of initial contamination.

On a few occasions there seemed to be more tainted than infected samples. In each case these samples, though apparently sterile, had a decidedly putrid taste and smell, which may have been due to the chemical changes resulting from the use of raw milk which was of a poor quality though free from heat-resistant spores.

(2) There was a sharp increase in the number of unsound bottles during the summer months. This is strikingly shown on the chart, where the sudden rise at the beginning of June is maintained until early in October, with the exception of a temporary fall from the middle of July to the middle of August, which cannot be wholly accounted for by low temperatures. It is noticeable that the results for the second winter were not so good as those for the first. The seasonal fluctuations are, as might be expected, most clearly marked in the curve showing the results of tests carried out at room temperature where changes in the atmospheric temperature would have the greatest influence. Unfortunately the cellar temperature also varied with the season, so that it was not possible to study the inhibitory influence of low temperatures on the spoilage of sterilised milk during the summer months. Very little seasonal fluctuation is shown by the curve for the bottles stored at 22° C., indicating that the increase in the spoilage from June to October is largely due to the rise in the prevailing temperatures of these months, a factor which favours the growth of infecting bacteria. But this does not altogether explain the increase in the degree of spoilage (stronger taints and more rapid coagulation) which was observed during these months but which cannot be shown on the curve. It would seem that the initial contamination is heavier during the summer months.

(3) The first section of the curves, November, 1925 to June, 1926, is interesting in that it shows that even though many of the samples were unsterile, the infection did not always result in coagulation or taint during the week of storage, especially in those held at the lower temperatures.

Types of spoilage and their associated bacteria.

This prolonged routine examination of bottles of commercial sterilised milk confirmed the observations made during the preliminary tests—that, as a general rule, the changes were of two distinct types, and that the characteristic bacteria associated with these changes could also be divided into two corresponding groups:

Group I. Organisms causing coagulation and digestion of the curd. These were found to be bacilli of the aerobic spore-bearing type which readily formed spores on solid media, gave rise to spreading colonies on agar plates, and induced an acid, alkaline or neutral reaction in litmus milk with subsequent digestion of the casein.

Group II. Organisms causing the development of a characteristic taint with no

visible change in the milk. These were bacilli which required special conditions for sporulation. They gave small, concrete or slightly spreading colonies on agar plates, and brought about a rapid bleaching of the litmus and a development of acidity in litmus milk.

In addition to these two well-defined groups a variety of other organisms were isolated from time to time. These included bacilli which produced an alkaline reaction but no digestion in milk, cocci and streptococci causing acidity and coagulation, and various organisms which brought about coagulation with the production of gas. These were, however, of infrequent and irregular occurrence and appeared to play only a minor part in the spoilage of commercial sterilised milk. They are, therefore, not discussed further in this paper, which is limited to the consideration of the two main groups of organisms mentioned above. The fact that the cocci are not, as a rule, highly heat resistant suggests that their presence in bottles of commercial sterilised milk was due to air infection as a result of faulty sealing.

The organisms of Group I comprised various species of bacilli. Many of these were isolated, but the number was too large to allow of detailed examination. A rough classification, mainly according to their reaction in litmus milk, was therefore undertaken and eventually two sub-groups were set up.

Group I. Sub-group A: contained organisms bringing about a more or less complete digestion of the milk, usually with a preliminary precipitation of the casein. The reaction was usually neutral or slightly alkaline—rarely acid. The majority of the organisms isolated during the preliminary routine studies belonged to this sub-group and two cultures 16*A* and 4*aw* were selected as being representative of the whole. A closer examination of these organisms and a comparison of their reactions with those of type cultures, has allowed of the identification of 4*aw* with *B. mesentericus vulgatus* (Flügge), and 16*A* has been shown to be very closely allied to, though not identical with, *B. cereus*. There are also certain points of resemblance between 16*A* and *B. mycoides*. The reaction in litmus milk is very much the same for *B. cereus*, *B. mycoides* and 16*A*, and as it was this reaction that was the chief basis for the original rough classification, it is probable that the sub-group of which 16*A* was selected as representative, included organisms of both *B. cereus* and *B. mycoides* types. It may be concluded, therefore, that the organisms of sub-group *A* consisted mainly of bacilli of the types *B. mesentericus*, *B. cereus* and *B. mycoides*.

Sub-group B: contained organisms bringing about an acid coagulation with subsequent contraction and slight digestion of the curd together with the separation of a clear whey. This type of change occurred less often, but it was of sufficient frequency to indicate that the causal organisms (represented by 32*B*) were important factors in the spoilage of commercial sterilised milk. The organism has not been identified, and it presents peculiarities which suggest that it is worthy of a closer study than has yet been possible. In the early stages of the work it was termed a "spore-bearer" and records were made of the production of spores. These early studies were, however, not made in very great detail, and

in the light of later findings it is now doubtful whether true spores were formed. Later investigations have shown that, during the development of this organism, clear, refractive bodies are formed within the cells. They retain the spore stain but are not liberated as free spores, and it is possible that it was these bodies that were originally described as spores. It is therefore not certain whether this organism once possessed the power of spore formation, but lost it during continued cultivation under artificial conditions, or whether it is incorrect to regard it as a member of the genus *Bacillus*.

Group II. The organisms of Group II presented many interesting features, and were accordingly subjected to closer examination. Their importance was emphasised by later experience of numerous epidemics of the "carbolic" or "coconut" taint for which they were responsible. A detailed account of these organisms is reserved for the second part of this paper.

Summary.

The results of a routine examination of samples of commercial sterilised milk extending over a period of 15 months are given and reviewed.

Three distinct types of spoilage have been found, and each of these has been shown to be induced by a definite group of bacteria.

Representative members of each group have been isolated and examined. The majority were found to be of the type *B. mesentericus*, *B. cereus* and *B. mycoides*. Two types have not been identified. Of these, one has been found to be the cause of the "coconut" or "carbolic" taint, and is more fully described in Part II of this paper.

PART II. THE "COCONUT" OR "CARBOLIC" TAINT. A STUDY OF THE CAUSAL ORGANISM AND THE FACTORS GOVERNING ITS SPORE-FORMATION

By M. I. CHRISTIAN.

It has been mentioned in the previous part of this paper that the most important type of spoilage which occurs in commercial sterilised milk is the "coconut" or "carbolic" taint. On this account the causal organism has been studied in greater detail than the others which were isolated during the routine examinations. This paper deals with the characters of the organism and more particularly with the conditions necessary for its spore formation.

The characters of tainted commercial sterilised milk.

If bottles of commercial sterilised milk are judged on outward appearance alone, those in which the "coconut" or "carbolic" taint has developed cannot be distinguished from those which are in good condition, since the presence of the taint is not associated with any visible change in the milk. At the same time the conditions of reduced pressure are unimpaired and it is only on opening the bottle, therefore, that the fault can be detected.

Infected milk is characterised by a strong and slightly acid smell which is reminiscent of the milk of coconuts. It is unpalatable, for not only is it acid and bitter, but it has a strong aftertaste which is generally described as "carbolic." The titratable acidity is equivalent to 0·03–0·04 c.c. of normal NaOH per 100 c.c. milk, and the *p*H varies from 5·5 to 6. This acidity is apparently insufficient to cause coagulation of the sterilised milk, but it readily clots on boiling.

Microscopical examination of the tainted milk invariably shows the presence of large numbers of spores and vegetative forms. The spores are large, ellipsoidal or kidney shaped—the vegetative forms, slender and granular. As has been seen in Part I of this paper, the inoculation of such milk into litmus milk brings about a characteristic reduction of the litmus with the formation of acidity without coagulation. On nutrient agar plates the organisms develop below the surface as small, compact colonies which tend to spread on continued incubation. These two reactions taken in conjunction with the formation of the taint have been considered to be typical of the organism.

The occurrence of the taint is by no means localised, and epidemics of varying severity have been recorded in many parts of the country. Comparison of the causal organisms from widely different sources shows, however, that no essential difference exists and it may be concluded that one type of organism only is involved.

Examination of the causal organism.

Cultures isolated during the routine investigations of 1926–7 were used as the basis for the detailed study of this organism. Several strains had been maintained in litmus milk from the time of isolation and a selection of them was purified by plating. On careful comparison these pure cultures were found to be indistinguishable from one another and one strain (*AB* 1) was, therefore, selected and made the subject of these researches.

Morphology. In 24 hours' old culture on nutrient agar the organisms are slender bacilli, $2{\cdot}25 \times 0{\cdot}75\,\mu$, occurring singly, in pairs or in short chains. The cell contents are typically granular. No spores are present.

Motility. Non-motile. No flagella have been demonstrated.

Staining properties. Gram-negative in nutrient agar culture.

Spore formation. In laboratory culture the organism exists in a stable vegetative form, and all efforts to induce sporulation in such cultures have proved unsuccessful. The relation between the spore form which is found in tainted commercial sterilised milk and the vegetative form of laboratory culture is dealt with later in this paper. In the sporing state endospores are formed in a slightly sub-terminal position in the organism. They are typically ellipsoidal or kidney-shaped and measure $2{\cdot}25 \times 1{\cdot}25\,\mu$.

Nutrient agar slope. After 24 hours' incubation at 37° C., growth appears in small discrete colonies, moist, transparent, with a bluish tinge. Later, the edges spread slightly and the colonies tend to coalesce to form a thin transparent layer which covers the surface of the agar. It is slightly slimy and easily detached from the surface of the medium.

Nutrient agar stab. There is good growth along the line of inoculation extending to the bottom of the stab. It is finely branched and arborescent. There is little surface growth.

Nutrient agar plates. When plated in the ordinary way the colonies invariably develop below the surface. After 24 hours' incubation at 37° C., or 48 hours at 22° C., they become just visible to the naked eye. On continued incubation they increase in size but never exceed a maximum diameter of 1·5–2 mm. In the early stages, under the low power of the microscope, they are round, oval or lens shaped with dense, yellow contents and entire edges. Later, at a point on the surface of the colony, the inner portion breaks out and begins to spread as a fan-shaped mass of transparent growth. This extended area (which is also confined to the depths of the agar), is composed of a thread-like mass which is suggestive of the "Medusa head" type of colony described by Wagner (6) and Preisz (7) for *B. anthracis* and by Soule (8) for his "Rough" variants of *B. subtilis*. The surface shows characteristic markings which are best described as "convoluted." On further development, the spreading area extends until it completely surrounds the central portion of the colony. In its final state the colony consists almost entirely of the transparent spreading mass, whilst all that remains of the original form is a small dense area at the centre.

When serial observations are made of the development of the colonies on surface streak culture, the dense or concrete forms of depth culture are absent. Spreading begins at once and the colony consists entirely of the transparent, convoluted material. Well-isolated colonies in these cultures may reach a diameter of 8–10 mm.

Dextrose agar plates and slope. Growth is similar to that on nutrient agar but less profuse.

Gelatine stab. Growth is good along the line of inoculation but of a very delicate character. In the early stages it tends to take the form of small discrete colonies, but later it spreads in short horizontal lines radiating from the line of inoculation. There is little or no growth on the surface, and no liquefaction.

Gelatine plates. After 7 days' incubation at 22° C. the colonies are small and dense each surrounded by a spreading zone. Under the low power of the microscope this spreading zone is seen to be composed of threads which radiate from the edge of the colony in rope-like strands. There is no liquefaction.

Lemco broth. In 24 hours a greyish flocculent precipitate appears. On being disturbed it extends upwards through the medium in long strands. There is neither turbidity nor pellicle formation.

Peptone water. In 24 hours a slight precipitate develops, but as in the case of broth, there is neither turbidity nor pellicle formation.

Potato (neutral and acid). There is no visible growth even on prolonged incubation. On scraping the surface, organisms can generally be recovered, but if growth occurs at all it is of a very reduced nature.

Litmus milk. After 12 hours' incubation at 37° C., a bleaching of the litmus occurs at the bottom of the tube. It extends upwards until at 24 hours the litmus has been completely reduced. At this stage the colour of the litmus returns, and acidity, as denoted by the development of a red colour, gradually extends from the surface downwards throughout the medium. Determinations of titratable acidity and *p*H during this period show that the acidity in milk is produced from the outset of growth, but in litmus milk culture its development is not recorded by the litmus owing to the excessive reducing action of the organism in the early stages. The reactions are complete by the 7th day. No coagulation or peptonisation takes place, and even after long periods of incubation cultures continue to show the marked acidity without any apparent physical change.

The reactions take place at all temperatures from 5° C. to 40° C. Total reduction of the litmus occurs on the 2nd day at 22° C., the 5th day at 15° C., and the 10th day at 5° C. A characteristic feature of all milk reactions, including litmus milk, is the production of the "coconut" or "carbolic" taint.

Blood serum. There is good growth of small transparent colonies which coalesce later as in the case of agar cultures. There is neither liquefaction nor discoloration of the medium.

Carbohydrates: (1) Bleaching of the litmus with the development of strong acidity occurs in inulin, dextrin, maltose, lactose, dextrose, galactose, laevulose and glycerol.

(2) Bleaching with slight acidity occurs in salicin, saccharose and raffinose.

(3) No reaction occurs in adonitol, sorbitol, dulcitol, inositol, mannitol and arabinose.

In no case is gas produced.

Thermal death-point. Vegetative cultures are destroyed by exposure to 60° C. for 5 minutes. The spores, heated in milk suspension, either in bottles of milk or in capillaries, are destroyed at 100° C. for 3 hours.

Oxygen requirements. Facultatively anaerobic.

Indol formation. Negative.

Reduction of nitrates. Nitrite reaction, negative; ammonia reaction, negative.

Acetyl-methyl-carbinol formation. Negative.

Catalase production. Positive.

Temperatures of growth. Growth takes place at all temperatures from 5 to 40° C. The optimum for vegetative growth appears to lie between 30 and 37° C. and for spore formation at 22° C.

Characters of the taint. The characteristic "coconut" or "carbolic" taint is produced in milk by both sporing and vegetative cultures of the organism and develops at all temperatures from 5 to 40° C. The taint-producing substance is steam-volatile and the distillates give positive ketone reactions by the potassium bisulphite method. These are probably methylamyl, methylheptyl and methylnonyl ketones produced by the degradation of the milk fat, and their mode of formation may be similar to that described by Stokoe (9) for the rancidity of coconut oil produced by *Pencillium palitans*. In 1930, Hiscox and Mattick (10), working with samples of commercial tainted milk and cultures of *AB* 1, demonstrated that the production of phenolic or cresolic substances was associated with the growth of the organism. Later, quantitative determinations by the author using the method of Folin and Denis (11) showed that on an average these substances were present in a concentration of four parts per million. It is probable that even these small quantities are sufficient to account for the "carbolic" aftertaste which is so characteristic of tainted milk. Bell (12) associated the production of phenols by bacteria with the degradation of tyrosine. It may be in the present case that the casein of milk provides the source for a similar degradation.

It will be seen from the foregoing characters that the organism is a facultatively anaerobic bacillus, but the specialised conditions which it requires for the formation of spores indicate that it is not a typical member of the group *Bacillus*. All attempts to induce the vegetative culture *AB* 1 to sporulate were unsuccessful, and had it not been that a spore form was known to exist under

commercial conditions, the organism might reasonably have been classed as a non-spore-bearer. There is no record to be found in the literature of a species with which *AB*1 may be identified, but it has many characteristics in common with the *Plectridium novum*[1] (*Bacillus novus*) which was isolated by Huss (5) in 1907 from tins of sterilised milk showing an imperfect pressure. The milk in this case was always found to be coagulated, and beyond a slightly acid and bitter taste there is little to connect it with the fault under consideration. The causal organism, however, shows certain similarities with *AB*1 which make it worthy of closer attention. It has not been possible to obtain a culture of *B. novus* for comparison, but so far as can be judged from the records of Huss, the morphology and growth of the two organisms in the laboratory media (with the exception of milk), are almost identical. There is close agreement in the temperature and oxygen requirements. The analogy from the point of view of the Gram-staining properties is rather less complete. Huss records for *B. novus* that "das Plasma der Oidien und der sporulierenden Stäbchen hält nur Spuren des Gentianaviolettes zurück. Es erscheint somit schwach blau tingiert weshalb man das *Plectridium novum* als Gramnegativ zeichnen kann." The present organism has never retained these traces of gentian violet, but it is possible that variations in technique may account for the difference. Huss continues: "Sowohl die allerjüngsten, terminal lokalisierten Sporenanlagen wie auch die reifen sporen sind Grampositiv." This has never been demonstrated for *AB*1.

There are two main points which suggest that the two organisms are not identical:

(1) *The reaction in separated milk. B. novus* at 37° C. and 20° C. forms a firm clot with slight acidity in 2 and 5 days respectively. With continued incubation the clotted milk becomes strongly acid, the casein is gradually precipitated and is to some extent dissolved. A culture after a month's incubation is almost neutral in reaction and has a distinctly bitter taste. With *AB*1, on the other hand, a rapid increase in acidity takes place, and by the 2nd day at 37° C. and the 4th day at 22° C., it is equivalent to approximately 0·03 c.c. of normal NaOH per 100 c.c. At the same time the characteristic "coconut" or "carbolic" taint is produced, but no coagulation occurs even after long periods of incubation.

(2) *Spore formation.* In *B. novus*, spores are produced with ease and the power of sporulation can apparently be maintained. The spore form of *AB*1 is unstable in laboratory culture, and unless certain definite conditions are observed the power of spore formation is rapidly lost. Full information is not given, but it is clear that Huss used, for his agar cultures at any rate, an inoculum of heated spores. If he used this as his standard inoculum throughout, and had no occasion to cultivate the organism through several generations of laboratory media, it may be that an irregularity in the process

[1] According to Bergey's classification (*Manual of Determinative Bacteriology*, Baltimore, 1923) *Plectridium novum* should be classified as *Bacillus novus*.

of spore formation corresponding with that which occurs in *AB*1 was overlooked.

A consideration of these points shows that the causal organism of the "coconut" or "carbolic" taint of commercial sterilised milk, while resembling *B. novus* in many respects, is not identical with it. The organism, therefore, remains unidentified, and as these investigations have failed to show any true analogy with a previously described species, it seems that a new species has been established. The question of its nomenclature will be dealt with more fully in a subsequent paper.

Spore formation.

Early in the course of the work two facts emerged:

(1) That under commercial conditions the organism existed in a spore form; and

(2) That under conditions of laboratory culture this spore form could not be demonstrated and the organism existed only in a vegetative form.

The cultivation of the vegetative culture *AB*1 on the normal sporulating media, *e.g.* agar and potato, failed to give spores. Efforts were, therefore, made to induce it to sporulate by other means. Conditions of enriched and impoverished media were tried: drying; varying conditions of pressure and oxygen tension; a wide range of temperatures of incubation; shock, in the form of heat, acid and alkaline solutions applied to vigorous cultures, but all failed to produce spores. These negative results were not without their value, however, for they showed at least that the vegetative form was a very stable one. But further work on these lines was discontinued, as it was realised that the conditions for sporulation must be of a highly specialised character, and attention was directed to the study of the spore form which could always be found in bottles of commercial sterilised milk showing the "coconut" or "carbolic" taint.

*Relation between the spores in tainted milk and the vegetative culture AB*1. Samples of commercial sterilised milk in which the typical spores could be demonstrated were heated at 100° C. for 30 minutes in litmus milk. On plating this material on nutrient agar and incubating at 22° C., an apparently pure culture of small and slightly spreading colonies developed. On comparing them with the colonies of corresponding cultures of *AB*1 it was found that, during the earlier stages at any rate, no essential differences existed. This observation was further strengthened by the fact that on inoculation into litmus milk, the colonies of both cultures gave the characteristic reactions with the production of the taint. Repeated plating and selection of colonies gave a culture which could be considered pure, but when this culture was examined microscopically it was found to consist of vegetative forms only and no spores could be demonstrated. Inoculations on sporulating media—agar slopes, potato and gypsum blocks—failed to give spores. The characteristics of the cultures were then examined in greater detail, and it became apparent that they were identical

with those of *AB* 1. It was possible, therefore, to conclude that, since the original inoculum had been heated at 100° C. for 30 minutes, the spore form present in the bottles was indeed related to the vegetative form of laboratory culture, but that the power of sporulation had for some reason been lost during the purification of the culture.

This work was repeated in nutrient agar slope cultures, and the transition from the spore form of the bottle to the vegetative culture of the laboratory, followed by microscopical examinations at regular intervals during eight serial transplants. The results of these examinations were significant. In the first transplant, the inoculum of heated spores germinated and large numbers of new spores were formed. At the same time, slender non-sporulating vegetative forms appeared in considerable numbers. On inoculating this material into the second generation, the proportion of vegetative forms showed a marked increase, and this time only a very small number of the spores germinated. By the third transplant, the number of spores had decreased still further and there was no evidence of the germination which had taken place in the previous generations. The fourth transplant was sufficient to bring about the disappearance of almost every spore, and by a fifth only the vegetative forms persisted. Continued culture up to the eighth generation failed to show any recurrence of spore formation, and the culture finally established itself as a vigorously growing vegetative culture identical with *AB*1.

These results were confirmed by the use of litmus milk and whole milk in place of nutrient agar, and again the spores were lost after the fifth transplant. It appeared, therefore, that during the cultivation of the organism in the laboratory, the spore form was rapidly replaced by an actively growing vegetative form, and like the culture *AB*1, this vegetative form must require very highly specialised conditions for sporulation.

The transformation of the spore form to the vegetative form had taken place so rapidly that it indicated that some factor in the laboratory methods was exerting an unfavourable influence on the spores. On the other hand, in infected bottles of commercial sterilised milk, the organism was invariably found to be present in the spore form, and while it was uncertain whether the site of sporulation was the farm, the plant or the bottle of milk itself, it seemed that at some point during the process conditions must exist which determined spore formation.

Propagation of spores in bottles of heated milk. The conditions of the bottle of milk were investigated first, and efforts were made to reproduce those of the commercial process as nearly as was possible in the laboratory. For this purpose, and for all the succeeding work with bottle cultures, a supply of Grade A (T.T.) milk from a single cow was selected and carefully tested to ensure its freedom from heat-resistant spores. This milk was invariably found to be sterile after heating at 100° C. for 30 minutes. Nevertheless, the accuracy of the experimental work was safeguarded by the use of liberal numbers of controls. Immediately after milking, the milk was filled into sterile swing-

stopper bottles of the type used in commerce. In order to avoid unnecessary handling of the milk and probable contamination, no effort was made either to pasteurise or homogenise the milk before it was filled into the bottles. This was a deviation from commercial practice, but it was proved later to have no influence on the results. A standard inoculum was used throughout and consisted of 1 c.c. of a suspension of 10 c.c. of a milk culture containing spores in 90 c.c. of saline. One c.c. of this material added to a bottle of milk was found to introduce an average of 277,000 spores capable of withstanding heating at 100° C. for 30 minutes. The bottles of milk were then steamed at 100° C. for 30 minutes with the stoppers placed loosely in the necks. On opening the steamer at the end of the heating, the bottles were sealed and allowed to cool at room temperature. When sufficiently cool, they were removed to incubators. Except where otherwise stated the temperature of incubation during the experimental work was 22° C. The use of this temperature was suggested by the fact that the epidemics of the taint occurred only in the summer. It will be shown later in this paper that the choice was a fortunate one, for it has been found to be the optimum temperature for spore formation.

In the present experiment incubation was continued for 7 days. On opening the bottles at the end of this time the milk was found to be acid and strongly tainted, and microscopical examination showed large numbers of the typical spores and vegetative forms. Plate counts of heated portions of this milk showed that the 277,000 spores which had been introduced into the bottle had increased to an average total of 775,000,000. Spore formation had therefore taken place in the bottle. This work was repeated many times, and it was finally established that *if tainted milk containing spores were inoculated into bottles of milk and heated as nearly as possible according to commercial practice, spore formation would take place.*

The further investigation of the conditions for maintaining spore formation resolved itself at this point into a study of the conditions in the bottle of sterilised milk. A comparison of the methods of propagation in the laboratory (*e.g.* in tubes of whole or separated milk) which resulted in the loss of the spores, with those of the bottle culture which maintained sporulation showed many points of difference. Chief among them could be reckoned the pressure and oxygen conditions, the volume of the medium and the heating process, but preliminary work sufficed to show that of these factors only the heating process was of real importance in spore formation.

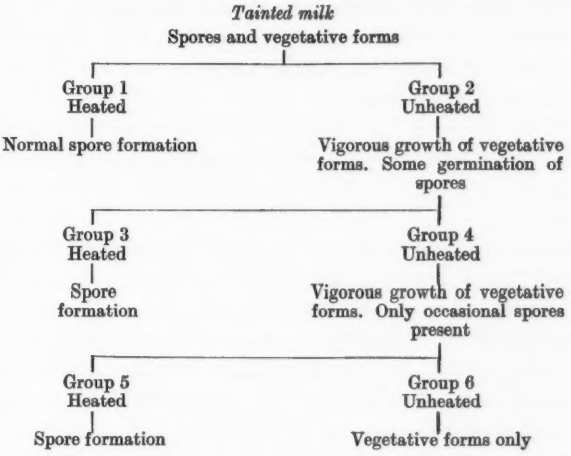
The heating process.

The earlier investigations of the influence of heating on spore formation were carried out in bottle culture.

A series of bottles of milk was divided into two groups. In the first of these (Group 1) inoculation and steaming were carried out in the usual way, but in the second (Group 2) the bottles were steamed first, and when cool, inoculated with equal portions of the same inoculum as had been used for Group 1

(Diagram 1). The whole series was incubated at 22° C., and bottles from the two groups were tested by microscopical examination after 3, 6, and 9 days.

Diagram 1. *The influence of the heating process on spore formation.*



Examination of Group 1 showed a normal spore formation. In Group 2, while some germination and resporulation occurred in the spores of the inoculum, the main feature of the culture was the rapid and vigorous growth of the vegetative forms. At the 9th day, ungerminated spores of the inoculum persisted in considerable numbers.

Using the material of Group 2 as inoculum (Diagram 1), the experiment was repeated, and, as before, half the bottles were treated in the ordinary way, while in the remaining half the milk was steamed and cooled before inoculation. These two groups have been called 3 and 4 respectively.

Microscopical examinations revealed a normal, if slightly reduced, spore formation in Group 3. In Group 4 the vegetative forms grew abundantly, and this time to such an extent that the culture soon consisted almost entirely of these forms. Occasional organisms were seen to bear small atypical spores, whilst only very few of the ungerminated spores of the original inoculum persisted.

When carried into a third series with the material of Group 4 as inoculum (Diagram 1) the heated culture gave, as in Group 3, a normal if slightly reduced spore formation. The unheated culture (Group 6) showed again the vigorous growth of the vegetative forms. Spores, however, were absent, and the culture consisted entirely of vegetative forms.

It became evident from this that the welfare of the spore in bottle culture was fundamentally bound up with the heating process. The spores had been lost in three generations when cultures had been propagated without the application of heat at the time of inoculation; but when the same cultures were

inoculated into bottles of milk and steamed in the ordinary way, the few spores present were able to germinate and resporulate. In order to prove that these results were due to the action of the heating process and independent of the physical conditions in the bottle, the work was repeated with litmus milk cultures in test tubes. The results were in complete agreement with those of the bottle cultures, but on account of the smaller volume of milk (10 c.c.) and a relatively heavier inoculation, the spores were not lost in the unheated cultures until the 5th generation.

These results, therefore, justified the conclusion that the heating process was a very important factor in maintaining spore formation.

It is evident that the heating of a typical sporing culture brings about fundamental changes involving primarily:

(1) the destruction of all vegetative forms;

(2) the liberation of their cell contents into the medium;

(3) the destruction of heat-labile products of growth, and

(4) the application of a stimulus to the spores which may be necessary to enable them to germinate.

Of these the most obvious is the destruction of the vegetative forms, but whilst this is undoubtedly of importance, the possible influence of a stimulus applied to the spores, or the removal of growth products of the vegetative forms cannot be overlooked.

As has been seen, the methods employed in bottle culture involve the application of heat immediately after inoculation. In this way the heating process induces in the added inoculum the changes which have been indicated above, and the spores are left in pure culture. If, however, the heating process is omitted, the inoculum is then no longer a pure culture of spores but a mixture of vegetative forms and spores. Microscopical examinations of the transition from the sporing to the vegetative race, in the unheated bottle cultures and in laboratory culture showed that the change is characterised by the rapid growth of the vegetative forms, and that, as the proportion of these forms increases, so the ability of the spores to germinate apparently decreases. These results can be interpreted if it is assumed that in the unheated culture there are certain factors which are unfavourable for the development of the spores. If, as seems possible, the inhibitory factors are associated with the actively growing vegetative forms, then the destruction of these forms by the heating process will release the spores from the inhibition and provide them with the conditions necessary for growth.

A series of experiments repeating the previous work, but designed to give quantitative results, was carried out in order to test the validity of the above suggestion.

In the first of these, typical sporing cultures in milk were plated in the ordinary way without heating, and again, after heating at 100° C. for 30

minutes. Comparative counts were made after 7 days' incubation at 22° C. The results of these counts are shown in Table I.

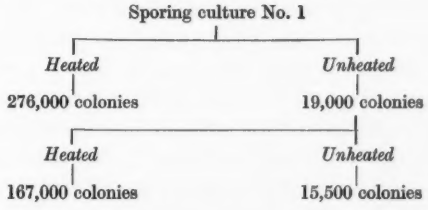
Table I.

Culture No.	No. of colonies per 1 c.c.	
	Heated	Unheated
1	276,000	19,000
2	1,080,000	17,600
3	235,000	34,500
4	343,000	22,600

It will be seen from this that the number of colonies which developed from the heated cultures was significantly higher than that from the same cultures unheated. All the colonies which developed from the heated culture had presumably arisen from spores. In the unheated cultures, on the other hand, both spores and vegetative forms had been present and the colonies therefore had equal chances of being of spore or vegetative origin. In spite of the fact that the presence of both spores and vegetative forms might be expected to give rise to relatively larger numbers of colonies, the count was actually much lower than that for the heated culture in which only spores were present. This indicated that only a small fraction of the total number of spores had developed.

The fact that functional spores were present in the unheated cultures although they had not developed as colonies was proved by further examination of the unheated culture No. 1 which had shown a count of *19,000*. One portion was plated unheated, another after heating at 100° C. for 30 minutes (Diagram 2). After incubating at 22° C. for 7 days, counts of *15,500* and *167,000* respectively were obtained.

Diagram 2. *Comparative counts obtained from heated and unheated portions of culture No. 1.*



This demonstrated that large numbers of spores were evidently present and fully functional in the unheated culture, but until heat had been applied, the conditions of the culture were inhibitory to their development.

In a second series of experiments the nature of the inhibition was more fully investigated.

Sporing cultures were again divided into two portions, and one was heated at 100° C. for 30 minutes. Five c.c. of the heated cultures were transferred to sterile tubes and 1 c.c. of the corresponding unheated cultures added. The

heated, unheated, and mixed cultures were then plated on nutrient agar in the ordinary way. The mixed culture was incubated at 22° C. for 24 hours and then replated. The comparative counts after 7 days' incubation are shown in Table II.

Table II.

	No. of colonies per 1 c.c. of culture			
			Mixed: 5 c.c. heated, 1 c.c. unheated	
Culture No.	Heated	Unheated	Plated immediately	Plated after 24 hours
2	1,080,000	17,600	393,000	19,600
3	235,000	34,500	126,000	86,000
4	343,000	22,600	250,000	49,200
Mean	552,700	24,900	256,400	51,600

These figures confirmed the previous results and showed clearly that the unheated cultures bore some factor inhibitory to the development of the spores. The potency of the factor was apparently great, for even the brief contact of the few minutes necessary for dilution and plating was sufficient to reduce the mean count from *552,700* to *256,400*, a reduction of almost 50 per cent. After 24 hours' contact, its influence had brought about so much inactivation of the spores that only *51,600* developed; and this number, while slightly higher, was clearly of the same order as the *24,900* which had been the original count of the unheated cultures. At least 501,100 spores had therefore been inactivated as a result of the 24 hours' contact.

These results indicate that the growth of the vegetative form is antagonistic to the development of the spores. It is evident that this antagonism may explain the disappearance of the spores in cultures propagated under laboratory conditions. In the absence of heating, and consequently in the presence of the inhibitory substance, little or no germination takes place after the first generation. Thereafter, the rapid predominance of the vegetative forms intensifies the inhibitory conditions, and the spores, being incapable of germinating under these conditions, are lost in succeeding generations by a process of dilution. The influence of the vegetative forms, while inhibitory to germination, is, however, not destructive to the spores themselves, for the spores, if recovered, will develop normally when placed under the correct conditions. It is worthy of note in this connection that Cowles(13) has recently reported an inhibition of germination in the spores of *B. megatherium*. The inhibitory agent in this case is bacteriophage.

There is little published work dealing with the germination of spores. It has usually been assumed that the removal of spores to favourable nutritional conditions leads naturally to their germination, and in many cases this may be so. But the use of heat has always suggested itself as an obvious method of procuring a pure culture of spores, and most of the information concerning spores has been gained from work done with heated cultures. Without being aware of the real importance of the heating process, workers may have provided

the spores with the essential conditions for growth, and thus the influence of the presence of the vegetative forms and growth products on germination has been overlooked.

Contemporary work by Mudge and Thorwaldson in America on the thermophilic bacteria in relation to pasteurisation indicates that they too have realised this fact. No full scientific account of their results has yet been published, but they state in preliminary notes(14, 15) that their evidence has gone to show that the dormancy of spores can only be broken by the stimulus of certain physical and chemical agencies, such as heat, cold and the application of alkalis. In a letter to *Nature* (April 11, 1931) the claims of Mudge and Thorwaldson are correlated more fully with the results of the experimental work which have been presented above.

RELATION BETWEEN SPORE FORMATION AND COLONY FORM.

Work which is now in progress shows that the spore form of this organism is unstable. During the process of spore formation it segregates into two types: (*a*) a sporogenic, which is represented by new spores, and (*b*) an asporogenic, which is represented by stable vegetative cultures (*e.g.* *AB*1). In surface cultures on agar, it has been found that these two types are associated each with a characteristic colony form. The sporogenic colony is round, opaque and refractive, and consists of a mixture of sporogenic and asporogenic individuals. The asporogenic colony is, on the other hand, spreading, convoluted and translucent, and is made up of vegetative forms only. These colony types appear to agree closely with the "Smooth" and "Rough" variants ascribed to *B. anthracis* and many other organisms. The growth of the asporogenic form has been found to be definitely antagonistic to that of the sporogenic. For the purposes of the present paper, however, this aspect of the work will not be considered further; it is mentioned merely to show that there is additional evidence to support the antagonism which is observed to exist between the vegetative forms and the spores in milk culture.

THE SPORE CYCLE.

With the conditions for spore formation established, closer examinations were made of the actual process. The preliminary work with bottle cultures had shown that sporulation was invariably complete by the 7th day. The later investigations were, therefore, confined to observations of the development of the organism at 24-hour intervals from the time of heating up to the 7th day. Bottle cultures were used, for although similar results could have been obtained in heated litmus milk cultures, the use of bottles of milk gave the work an additional value from the commercial point of view.

The method of testing consisted in opening the bottle and removing 5 c.c. of the milk. After centrifuging, smears were made from the deposit and stained by Moeller's spore stain method. The remainder of the milk was used for the detection of the taint, determinations of the titratable acidity and *p*H, and

reaction to the boiling test. The tests of taste and smell had to be relied on throughout for the detection of the taint as no rapid quantitative method was available, but the personal factor was reduced to a minimum in that the tests were always made by the same person. By comparison with controls and known standards, the degree of accuracy attained was sufficiently high for the purposes of this work. The titratable acidity was determined by titrating duplicate quantities of 25 c.c. with $N/10$ NaOH, using phenolphthalein as indicator. The *p*H measurements were made by means of the quinhydrone electrode.

The results of these investigations are shown in Table III.

Table III.

Age of culture (days)	Taint	Clot on boiling	Titratable* acidity	*p*H	Morphology
1	–	–	0·0176	6·52	Germination
2	+	–	0·0231	6·24	Active vegetative growth
3	+++	–	0·0264	5·89	Sporulation—young spores within mother cells—granulated vegetative forms appearing
4	++++	+	0·0313	5·87	Spores maturing—vegetative forms increasing
5	++++	+	0·0335	5·84	Liberation of spores—vegetative forms increasing
6	++++	+	0·0360	5·71	,, ,,
7	++++	+	0·0371	5·66	,, ,,
14	++++	+	0·0416	5·50	Free spores and granulated vegetative forms in approximately equal numbers
21	++++	+	0·0455	5·44	,, ,,
Controls (mean values) 1–21 days			0·0181	6·55	Sterile

* Expressed in terms of c.c. normal NaOH per 100 c.c. milk.

It will be seen from the microscopical observations recorded in column 6 that the interval between the germination of the spores and the formation of new spores is approximately one day, *i.e.* the spore cycle at 22° C. takes 3 days. The significance of this in practice is considered more fully in the third part of this paper.

A characteristic feature of the spore cycle in this organism is that, coincident with the formation of the spores, vegetative cells appear which closely resemble the granulated forms so typical of the vegetative cultures, *e.g.* *AB*1. It is significant that these forms are first observed on the 3rd day whilst the young spores are still within the mother cells. No explanation is offered at present for the mode of their formation, but evidence goes to show that it is these individuals which ultimately form the asporogenic cultures. This observation may be of importance in interpreting the two distinct colony forms which arise in agar culture.

The formation of the taint with its associated increase in acidity takes place from the outset of growth, *i.e.* during the period of active vegetative reproduction. The greatest changes occur, therefore, during the period prior to the

formation of spores, and after the spores are formed very little further change can be observed. The formation of the taint is in no way dependent upon the formation of spores, since it is produced with equal readiness by the asporogenic cultures.

INFLUENCE OF TEMPERATURE OF INCUBATION ON THE SPORE CYCLE.

The influence of temperature of incubation on the spore cycle was investigated in bottle culture. The cultures were prepared in the usual way and groups incubated at 5, 15, 22, 30, 37 and 56° C. The development of the organism was followed by regular microscopical examinations and a résumé of the results is given in Table IV. Table V shows the corresponding development of the taint at these temperatures.

Table IV.

Time of incubation (days)	Temperature of incubation (° C.)					
	5	15	22	30	37	56
1	—	—	Germ.*	Germ.	Germ.	Gradual germ.— no sporulation
2	—	Germ.	—	—	—	
3	—	—	Spores formed	Abortive spores, occasional true spores	Abortive spores, no true spores	
4	Germ.	—	—	—	—	
6	—	Spores formed	—	—	—	
15	Spores formed	—	—	—	—	

* Germ. = Germination.

Table V.

Time of incubation (days)	Temperature of incubation (° C.)					
	5	15	22	30	37	56
1	−	−	−	+	++	−
2	−	−	+	+++	++++	−
3	−	?+	+++	++++	++++	−
4	−	+	++++	++++	++++	*?+
5	−	++	++++	++++	++++	?+
6	?+	+++	++++	++++	++++	?+
15	+++	++++	++++	++++	++++	+

The concentration of the taint is denoted as follows:

\+ = slight taint
++ = distinct taint
+++ = strong taint
++++ = very strong taint.

The horizontal lines indicate the points at which the milk clotted on boiling.

A consideration of these observations shows primarily that temperature has a very marked influence on the production of spores in this organism. At 5 and 15° C. the course of the cycle is similar to that at 22° C., but as might be expected, the process is slower requiring 15 and 6 days respectively for its completion as compared with 3 days at 22° C. The use of low temperatures, therefore, does not inhibit the development of the organism in infected milk, but the fact that it retards the process at all is of importance since, as will be

seen in Part III, it forms a natural controlling influence on the occurrence of the taint in commerce during the winter.

Temperatures above 22° C. have an inhibitory effect on spore formation. At 30° C., where only occasional spores are formed, and at 37° C., where no spores are formed, development takes the form of a rapid vegetative growth. The organisms on the 2nd day of incubation show evidence of an effort at spore formation. The cell contents concentrate in a terminal position as in the case of the formation of true spores, but no spore wall is formed. Later, these terminal bodies or "abortive spores" are liberated and persist in the cultures in the free state for long periods. It has not yet been demonstrated whether they are capable of developing further under suitable conditions or of giving rise to new generations of the organism.

56° C. is unfavourable not only to spore formation but also to growth. Examinations up to 21 days show that only a very gradual germination of the spores takes place during this period. The small numbers of vegetative forms which result are atypical and no subsequent sporulation can be demonstrated.

It may be concluded from these results that temperature of incubation is of considerable importance in spore formation. Its full significance, however, can only be realised when the results of these experiments are applied to the conditions of commerce. This aspect of the work is considered in greater detail in Part III of this paper.

Table V shows that the taint is formed at all temperatures from 5 to 56° C. If these results are examined in connection with the foregoing observations on spore formation, it will be seen that further evidence is afforded that the development of the taint is independent of spore formation. At 37° C., for example, where no spores are formed, a very strong taint is produced in 2 days, whilst at 22 and 15° C., where sporulation takes place, the most rapid development of the taint occurs before the spores are formed. This accords well with the view that it is the vegetative forms which are directly concerned in bringing about in the milk the chemical changes which constitute the taint.

Summary.

The characters are given of the organism causing the "coconut" or "carbolic" taint of commercial sterilised milk. Although certain similarities with *B. novus* (*Plectridium novum*) Huss are shown to exist, the organism remains unidentified and the question of its identity has been reserved for a subsequent paper.

In laboratory culture the organism exists in a stable vegetative form which has resisted all efforts to induce it to sporulate. Under commercial conditions, on the other hand, it occurs in a spore form in the bottles of tainted sterilised milk.

Propagated by ordinary laboratory methods, the spore form is rapidly replaced by a vegetative race. Five serial transplants are sufficient to bring about this transformation.

Sporulation can be induced and maintained indefinitely if sporing cultures

are heated at 100° C. for 30 minutes at the time of inoculation. The function of the heating process appears to be the destruction of a growth product inhibitory to the germination of the spores. This substance is associated with the growth of the vegetative form and its presence in unheated cultures is inhibitory to the germination of the spores.

Spore formation takes place at temperatures below 22° C., the spore cycle occupying 3 days at 22° C., 6 days at 15° C., and 15 days at 5° C. At higher temperatures, *e.g.* 30 and 37° C., it is inhibited and the normal process is replaced by an abortive effort at spore formation. The optimum temperature for the propagation of the spores is 22° C.

The author wishes to acknowledge the help which Dr W. L. Davies of the National Institute for Research in Dairying has given in the study of the chemistry of the taint.

PART III. THE BASIS OF CONTROL

By E. R. HISCOX and M. I. CHRISTIAN.

In the foregoing parts it is indicated that great difficulty may be experienced in the control of the spoilage of commercial sterilised milk which results from the development of spore-bearing bacteria.

A detailed study of the actual sources of contamination is now being made, but in the meantime it may be pointed out that many of these bacteria are typical members of the soil flora. They may therefore enter the milk from the dust either at the farm or the factory. The greater the care with which the milk is handled from the time of milking until the end of the heating process, and the greater the efficiency of the cleaning and sterilisation of the plant and the empty bottles, the less will be the danger of spoilage arising from the use of infected milk. During the course of the investigations recorded it has been necessary to carry out numerous control experiments. The milk used has been Grade A (T.T.) milk and the utensils with which it came into contact and the empty bottles into which it was poured were clean and *sterile*. There has never been the least difficulty in obtaining a *sterile*, fresh-smelling and sweet-tasting product after heating at 100° C. (212° F.) for 30 minutes and storing at 22° C. (72° F.).

Once the spores have been allowed to gain access to the bottles of milk the amount of heating to which they are subsequently subjected in practice is insufficient to destroy them. The spores of all the types examined were found to resist readily the process of heating which is still most widely adopted in England, *i.e.* at a temperature of 100° C. (212° F.) for 30 minutes. The spores of the organism of the "coconut" or "carbolic" taint were especially resistant, and it was found that heating at 100° C. (212° F.) for 3 hours was necessary for their destruction. Even an exposure to a temperature of 110° C. (230° F.) for 40 minutes was insufficient to kill them; it merely temporarily checked their growth.

The reduced pressure in the bottles exerts a certain amount of control over some forms of spoilage, but even when the sealing process is most efficient, and a "good vacuum" is obtained in the bottle, the growth of the taint-producing organism is not appreciably affected and that of 32 *B* is only slightly retarded. On the other hand, the growth of 16 *A* and 4 *aw* (the representatives of the *B. mesentericus-cereus-mycoides* sub-group) is greatly retarded by this reduction of pressure, but if the sealing process is faulty, these organisms are able to develop quite readily.

A great deal of spoilage may be prevented by suitable methods of storage, since the growth of 16 *A* and 4 *aw*, the types which most frequently occurred in the samples of commercial sterilised milk, is inhibited at 5° C. (40° F.). Unfortunately, however, 32 *B* develops slowly and the taint-producing organism comparatively readily at this temperature. All types grow well at 22° C. (72° F.).

It will be seen that although the spores may not be destroyed during the heating process, it is possible to reduce the amount of spoilage due to the action

of the aerobic spore-bearing types by means of efficient sealing of the bottles and storage at low temperatures. It is, however, impossible to control the development of the "coconut" or "carbolic" taint by these methods, and the eradication of this trouble presents special difficulties. The problem is complicated by the fact that a new generation of spores is formed actually in the bottles of infected milk. It has been shown in Part II that for the organism causing this taint the heating process is essential for the germination of the spores and subsequent resporulation. The commercial process of sterilisation, therefore, provides the conditions necessary for the proliferation of the spores. The bottles of tainted milk which are returned to the factory constitute a real source of danger, and the factory becomes the seat of the multiplication and accumulation of spores. At the farm, where there is no heating process, this proliferation does not occur and the possibility of infection of the milk with spores there is negligible compared with the possibility at the factory. During an outbreak of this trouble the factory itself becomes the chief centre of infection. The empty bottles, and through them the bottle-washing plant, act as carriers of the infection which may spread so rapidly that the trouble quickly assumes the proportions of an epidemic.

It has been shown that the growth of this organism cannot be effectively controlled in the bottles of milk, but the spores can be destroyed in the empty bottles by means of prolonged heating. The stage of the process immediately before the filling of the bottles with milk is, therefore, the point at which control measures can be most conveniently applied. Thorough sterilisation of the empty bottles by steaming at a temperature of 100° C. (212° F.) for a minimum of 3 hours has proved to be effective in practice. Work which is in progress indicates that chemical treatment, if it is to ensure sterilisation of the bottles, must be so drastic that it cannot generally be recommended as a substitute for steam sterilisation.

It is a characteristic feature of this trouble that it occurs mainly during the summer months. Its incidence is usually first recorded towards the end of May, and it appears to die away naturally in the autumn, *i.e.* in September or October, even though it may have been rife during the intervening months. Serious spoilage from this cause is confined to the summer and has never been recorded during the winter period. This may, to some extent, be due to a seasonal decrease in the amount of infection, but the fact that during the routine examination recorded in Part I this organism was detected in the samples of milk at all times of the year, indicates that the milk is always liable to infection. The rise and fall of the epidemics appears to be governed by prevailing temperatures.

At "average summer temperature," 22° C. (72° F.), spore formation is at an optimum. The germination of the spores, the subsequent rapid vegetative growth, and the eventual formation of a new generation of spores take place within 3 days at this temperature. The vulnerable period, *i.e.* the period during which the organism exists in the non-resistant vegetative form, is therefore

short, and, allowing for variations in the rate of germination of individual spores, it becomes almost impossible to find a period in the cycle when no spores are present. During the summer, therefore, practically every bottle which has contained infected milk contains spores when it is returned to the factory, and unless these spores are destroyed they will be the cause of immediate and widespread infection. Such an influx of spores would readily account for the sudden sharp rise which is so characteristic of these epidemics.

It has been recorded in Part II that the effect of lower temperatures of incubation is to increase the length of the spore cycle. For instance, at 15° C. (60° F.) it is 6 days, and at 5° C. (41° F.) it is approximately 15 days. It will thus be seen that the vulnerable period is greatly extended during the colder weather. Unless, therefore, there is undue delay in the return of the bottles to the factory, it is probable that in the majority of cases the spore cycle will be incomplete when they are received, and the milk residues will contain only vulnerable forms. These will be destroyed by the methods ordinarily adopted for the cleaning of the bottles and the processing of the milk, even when no sterilisation of the empty bottle is attempted. Although no special precautions may have been taken at the factory, the lower temperatures of the winter season act as a natural control, and probably determine the characteristic disappearance of the trouble with the approach of winter.

As a corollary to these facts it appears that the period which elapses between the time of the heating process and the return of the empty bottles to the factory should be as short as possible. It is particularly dangerous to carry large stocks during the summer. Whilst, however, a reorganisation of the system of distribution may have considerable value in lessening the amount of potential infection, it should be regarded merely as a subsidiary control measure which can in no way replace the sterilisation of the empty bottles. These represent the most important source of infection and it is to them that remedial measures can be most conveniently and effectively applied.

REFERENCES.

(1) Pellet (1928). *Le Lait*, **8**, 13, 108.
(2) Flügge (1894). *Z. f. Hyg.* **17**, 272.
(3) Weber (1900). *Arb. Gesundh. Amt.* **17**, 108.
(4) Hueppe (1891). *Berl. Klin. Wochenschr.* **28**, 717.
(5) Huss (1907). *Cent. f. Bakt.* II, **19**, 256, 420.
(6) Wagner (1920). *Ibid.* I, Orig. **84**, 386.
(7) Preisz (1911). *Ibid.* I, Orig. **53**, 510.
(8) Soule (1928). *J. Inf. Dis.* **42**, 93.
(9) Stokoe (1928). *Biochem. J.* **22**, 80.
(10) Mattick (1930). *Analyst*, **55**, 34.
(11) Folin and Denis (1915). *J. Biol. Chem.* **22**, 305.
(12) Bell (1921). *J. Inf. Dis.* **29**, 424.
(13) Cowles (1930). *J. Bact.* **20**, 15.
(14) Mudge (1930). *Proc. Soc. Exp. Biol. and Med.* **28**, 202.
(15) Mudge and Thorwaldson (1930). *Milk Dealer*, Dec. 1930, p. 57.

(*MS. received for publication, August* 11, 1931.)

34. STANDARDISATION OF MEDIA IN THE ACID RANGES WITH SPECIAL REFERENCE TO THE USE OF CITRIC ACID AND BUFFER MIXTURES FOR YEAST AND MOULD MEDIA

By J. G. DAVIS, M.Sc., Ph.D.

The National Institute for Research in Dairying, University of Reading.

(With 4 Figures.)

Introduction.

The importance of the adjustment of bacteriological media to a definite *p*H is generally conceded and for special purposes, such as the enumeration of yeasts and moulds in butter, it is essential. It is usual for this purpose to use beer wort or malt agar adjusted to *p*H $3{\cdot}5 \pm 0{\cdot}1$ (1). In the course of media preparation some difficulty was experienced in securing a final *p*H which fell within these limits and the work detailed below was carried out to overcome the difficulty.

Commercial media designed for growing yeasts and moulds to the exclusion of other micro-organisms require the addition of lactic acid to bring the *p*H to the correct level, and it was found that the addition of the stated quantity did not in all cases result in a *p*H of $3{\cdot}5 \pm 0{\cdot}1$. A further criticism lies in the fact that the lactic acid used requires careful standardisation before use. It was also found that colorimetric methods of adjustment in this range are liable to considerable error because brom phenol blue, the indicator usually employed, often fails to match correctly.

Methods of *p*H adjustment.

An attempt was made therefore to standardise a simple and convenient method of adjusting media, such as beer wort and whey, to a *p*H of $3{\cdot}5 \pm 0{\cdot}1$ by the preparation of titration curves with various acids. It is only possible, however, to use such curves for a medium of constant composition. Since beer wort and whey from different sources vary in composition the error involved in the use of such curves may be considerable. This is especially the case if the acid used does not buffer at the *p*H required (Fig. 1). This error may be minimised by the choice of suitable acids for adjustment.

Acids tend to stabilise the *p*H of their solution, *i.e.* to buffer most, when the

*p*H of their solution equals the *p*K of the acid. These terms are defined in the following equations:

$$p\mathrm{H} = \log\left(\frac{1}{\mathrm{H}^{\cdot}}\right),$$

where $(\mathrm{H}^{\cdot})$ = hydrogen-ion concentration in g./l.,

$$p\mathrm{K} = \log\frac{1}{K},$$

where $K = \dfrac{(\mathrm{H}^{\cdot})\,(A')}{(HA)}$, A' being the dissociated acid radicle and HA the undissociated acid. If the acid is partly neutralised (as in the case of an acid medium)

$$a = \frac{(\text{salt})}{(\text{acid}) + (\text{salt})}$$

(assuming that the salt is completely dissociated), where a = the degree of dissociation $= \dfrac{(A')}{(A') + (HA)}$.

From these may be obtained the following equation:

$$p\mathrm{H} = p\mathrm{K} + \log\frac{a}{1-a}.$$

Now $$\frac{d\,p\mathrm{H}}{da} = \frac{1}{a} + \frac{1}{1-a} \text{ and } \frac{d^2\,p\mathrm{H}}{da^2} = -\frac{1}{a^2} + \frac{1}{(1-a)^2}.$$

It follows that when $a = 0{\cdot}5$, $\dfrac{d\,p\mathrm{H}}{da}$ is at a minimum, *i.e.* when half the acid is neutralised the system has the greatest buffering power, and in addition $p\mathrm{H} = p\mathrm{K}$. Hence at this point the addition of acid or alkali (*i.e.* alterations in the value of a) has the least effect on the *p*H, or the solution tends to resist most strongly a change in hydrogen ion concentration. This resistance is a measurement of the buffering power of the solution and may be expressed as the number of c.c. of N acid or alkali required per litre to change the *p*H by unity at any particular *p*H. Hence when adjusting a medium the acid should, if possible, have its *p*K value equal to the *p*H required. Of acids having *p*K values from 3 to 4 the following appear to be the most suitable:

Citric acid (tribasic)	$p\mathrm{K1} = 3{\cdot}08$, $p\mathrm{K2} = 4{\cdot}39$, $p\mathrm{K3} = 5{\cdot}49$.
Lactic acid	$p\mathrm{K1} = 3{\cdot}86$.
Tartaric acid (dibasic)	$p\mathrm{K1} = 2{\cdot}96$, $p\mathrm{K2} = 4{\cdot}14$.

These acids are non-toxic and also serve as a good source of carbon for yeasts and moulds. Titration curves of these three acids with whey, and with beer wort diluted with an equal volume of water as used for laboratory media, were therefore prepared (Figs. 1 and 2).

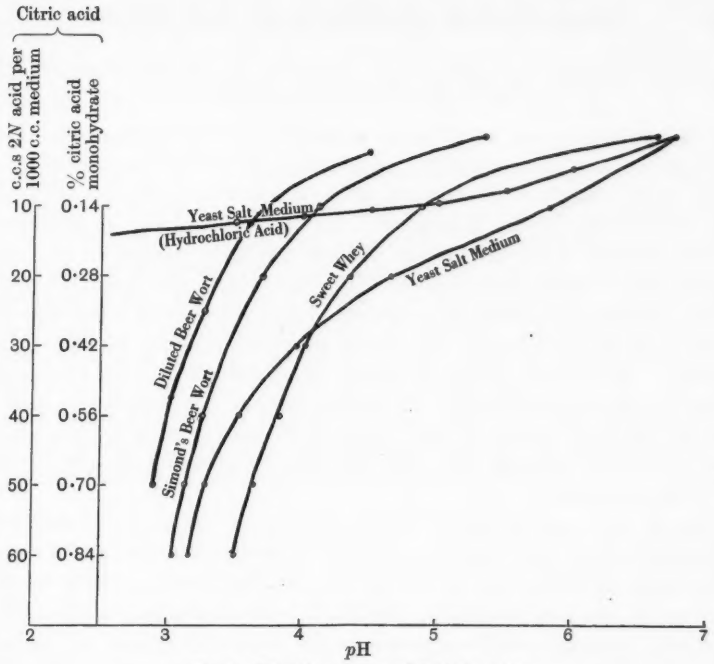


Fig. 1. Titration curves with citric acid.

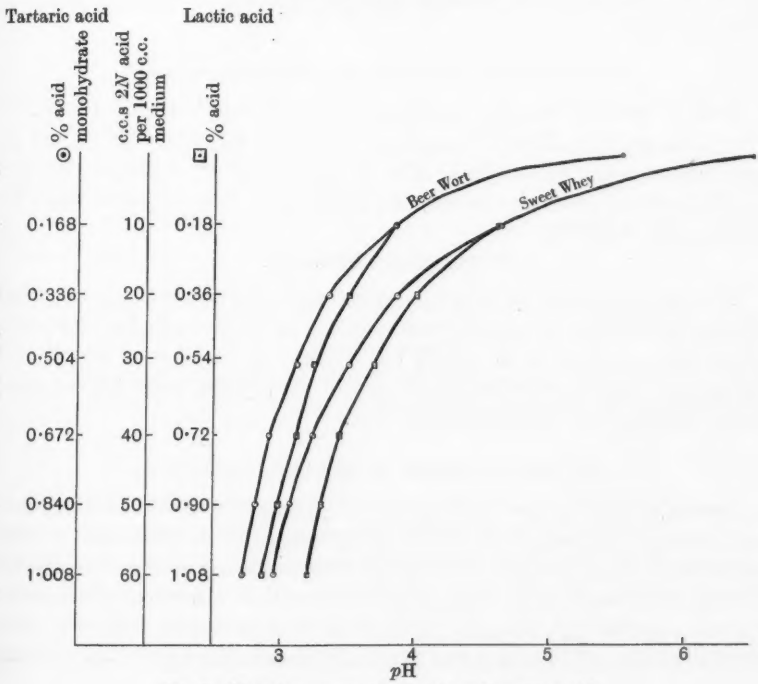


Fig. 2. Titration curves with lactic and tartaric acids.

Choice of acid for adjustment.

Citric acid was found to be the most suitable acid for adjustment. Its advantages are that it can readily be obtained pure, buffers over a broad range, forms readily soluble alkali salts and can be weighed out as the dry solid. It is only very slightly inferior to lactic acid from the point of view of buffering at *p*H 3·5. Owing to the insolubility of some citrates a precipitate invariably appears when a medium is adjusted with citric acid. In the normal course of media making this precipitate is filtered off; its removal does not appear to affect appreciably either the efficacy of the medium or the accuracy of adjustment. Lactic acid, however, is only obtainable as a syrup of unknown strength and requires dilution and standardisation, before it can be used for the adjustment of media. Tartaric acid can be weighed as the dry solid, but forms acid salts which are only sparingly soluble. It cannot therefore be used for buffer mixtures.

Finally potassium citrate, $K_3C_6H_5O_7 . H_2O$ (2 per cent.), when incorporated in standard media was found not to inhibit the growth of a variety of organisms (*Alcaligenes*, *Escherichia*, *Proteus*, *Serratia*, *Bacillus subtilis*, a pink torula, a white torula and a spore-forming yeast) and hence media for general as well as for special purposes can be adjusted by the method described in this paper *which does not require the standardisation of any acid or alkali or the electrometric determination of* *p*H. The only operation required is weighing, accurate to 0·1 per cent.

Preparation of cleared diluted beer wort.

Wort as received from the brewery was immediately heated to 100° C. for 1 hour. It was then filtered through pulp, allowed to cool to 20° C. and an equal volume of tap water added. After thorough mixing the medium was put into flasks (usually one litre in size) and sterilised by steaming three times for half an hour at 100° C.

Preparation of whey.

Whey was prepared by rennet coagulation of milk at about *p*H *c.* 6. After withdrawal from the vat the whey was adjusted to *p*H 5 colorimetrically (using methyl red) and heated at 100° C. for 1 hour. It was then slowly filtered through filter paper, the filtrate adjusted to *p*H 7 (using brom thymol blue) and re-filtered if any precipitate appeared.

Method of obtaining titration curves.

Quantities of 100 c.c. of beer wort or whey, prepared as described (without agar), were pipetted into beakers that had been in use for some time and various quantities of 2*N* acid added. The liquids were mixed and allowed to stand for 3 hours (addition of water does not affect the *p*H of a weak acid-salt buffer mixture appreciably). The *p*H values were then measured with the quinhydrone electrode (2, 3), which was frequently standardised against a standard

($M/20$) potassium hydrogen phthalate buffer solution at pH 3·97. The curves so obtained are given in Figs. 1 and 2.

SOURCES OF ERROR IN USING THE QUINHYDRONE ELECTRODE WITH MEDIA.

When determining the pH of beer wort and whey it was found that, using a fresh sample of solid quinhydrone, an equilibrium potential was obtained in 3 minutes. This potential remained constant for 30 minutes or longer. When using an old sample of solid quinhydrone, however, not only was a high initial value obtained but the potential fell only slowly to the value obtained with the fresh quinhydrone (Fig. 3). Moreover, the end point in the latter case was much less sharp than when using the fresh quinhydrone.

It is therefore important to observe two conditions, (1) not to take a final reading in less than 3 minutes from the time of mixing the quinhydrone with the medium and (2) to use a fresh sample of quinhydrone.

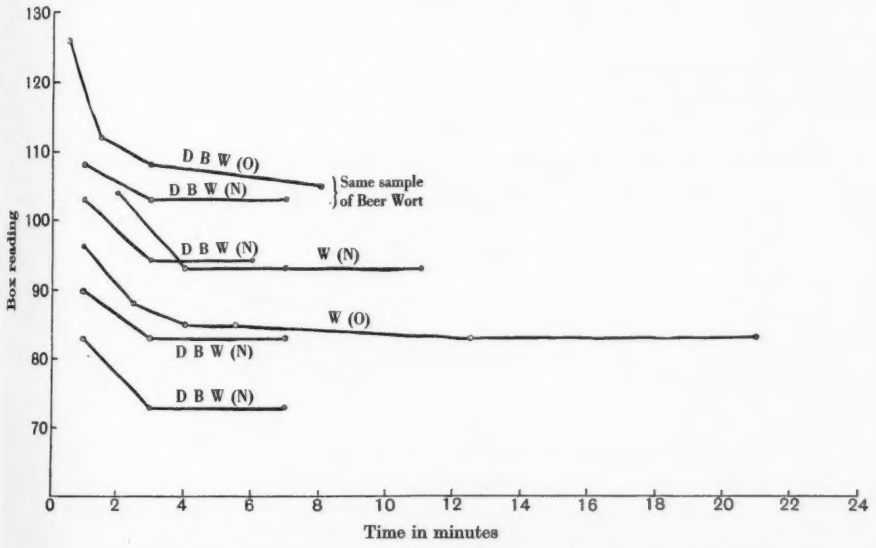


Fig. 3. Time-potential curves obtained with the quinhydrone electrode.
DBW = diluted beer wort; W = whey; (N) = new quinhydrone; (O) = old quinhydrone.

SOURCES OF ERROR.

In practice it has been found that media after tubing, subsequent to the addition of the acid, attain a slightly higher pH than would be indicated by the titration curve. This is a common experience with those who have adjusted media, and is no doubt due partly to the extraction of soda from the glass tubes by the hot acid medium, since small differences in the pH of individual tubes of the same batch have been observed. It is probable also that a constant

*p*H is not attained for some time on account of the colloidal nature of some of the buffering substances present. Agar which has been well washed has been found to be without any appreciable effect upon the *p*H of well buffered media, such as beer wort and whey.

The most serious errors, however, are those due to variations in composition of different batches of media and of media from different sources. Thus, in the case of worts from different breweries, errors up to 0·3 *p*H were found when using the citric acid value given by the titration curve for Simonds' beer wort. Attempts have been made to solve this problem in two ways:

(1) By incorporating a buffer system in the medium.

(2) By the use of a standard medium in which substances of variable composition are present only to a small extent.

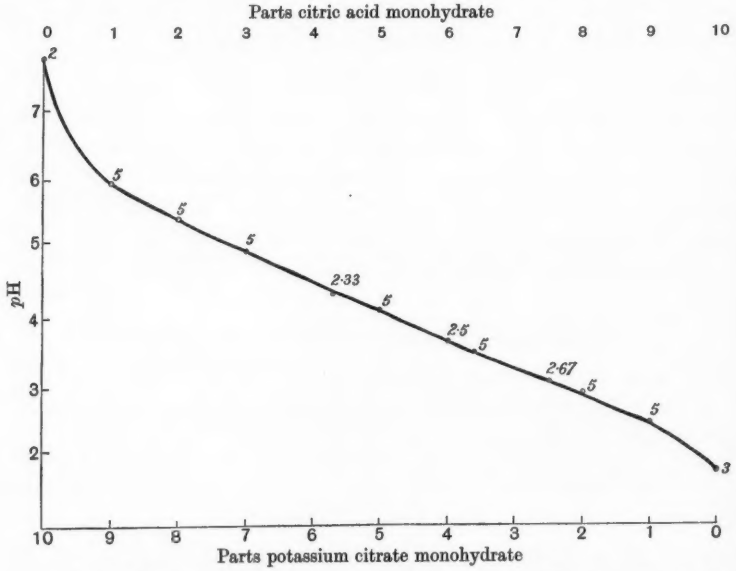


Fig. 4. Buffer curve of citric acid with potassium citrate. (The figures represent the percentage concentration of the buffer.)

(1) *The incorporation of a buffer system in wort and whey media.* Of possible buffer systems for *p*H 3·5 a citric acid-potassium citrate mixture appeared to be the most suitable. The values for this system were therefore determined (Fig. 4). Solutions of 5 per cent. citric acid monohydrate and 5 per cent. potassium citrate monohydrate were prepared, and the *p*H values of their mixture in different proportions determined with the quinhydrone electrode (Fig. 4). Between *p*H 2 and *p*H 6 there is practically a linear relation between the *p*H and the percentage of any one of these constituents. A ratio of 64 parts of citric acid monohydrate and 36 parts potassium citrate monohydrate gives

a buffer with *p*H 3·5. Wort and whey media were prepared, (*a*) with the addition of the theoretical amount of citric acid and (*b*) with 2 per cent. buffer mixture in addition to this required amount of acid, and the *p*H of these media measured. The results obtained with various samples of media are given in Table I.

Table I.

Medium	Amount of citric acid hydrate ($C_6H_8O_7H_2O$) added %	*p*H	*p*H found on addition of 2 % of buffer mixture
Fresh beer wort	0·35	3·50	—
" "	0·38	3·58	3·51
Diluted filtered beer wort	0·38	3·28	3·48
" " "	0·35	3·15	—
" " "	0·175	3·56	—
" " "	0·175	3·62	—
Fresh whey	0·84	3·50	—
"	0·84	3·66	3·48
Filtered whey	0·84	3·60	3·52
" "	0·84	3·45	—

The results obtained with the use of this buffer show that even with some considerable error in the adjustment of the medium, accuracy to within the limits required (*p*H 3·5 ± 0·1) may be obtained.

(2) *The use of a standard medium.* The following medium was devised to fulfil the requirements of a synthetic medium as closely as possible:

Yeast-salt medium.

	%
Ammonium nitrate	0·1
Ammonium sulphate	0·1
Dipotassium hydrogen phosphate	0·4
Potassium dihydrogen phosphate	0·2
Sodium chloride	0·1
Dextrose	0·1
Marmite	0·1
Agar	2·0

The *p*H of this mixture was found to be 6·6 without citric acid and 3·5 when 0·57 per cent. of citric acid monohydrate was added.

The only substance present in this medium which is not of definite composition is the marmite (or yeast extract) which is present to such a small extent that variations in its acidity will not appreciably affect the *p*H of the medium. Hence this medium, with citric acid, may be relied upon to have a *p*H of 3·5 ± 0·1 without adjustment or the inclusion of the buffer. Yeast and mould counts with this medium have been found to approximate very closely to those obtained with other media (Table II).

Preparation of media for yeast and mould counts. The volume of medium (prepared as already described) is measured before sterilising. The loss in water due to autoclaving amounts to about 5 per cent. and does not seriously affect the result. The required amount of acid (with the buffer, if this is to be

Table II. *Agar media at* pH 3·5.

	Bacto malt	Beer wort	Whey	Dextrose	Synthetic
Butter E.	8,000	4,000	—	5,000	6,000
	8,000	10,000	—	9,000	6,000
	7,000	11,000	—	1,000	7,000
Butter M.V.S.	—	230,000	—	206,000	195,000
	226,000	206,000	—	135,000	171,000
	290,000	—	—	183,000	214,000
Butter F.	320,000	280,000	270,000	—	270,000
	72,000	94,000	70,000	—	117,000
	210,000	195,000	198,000	—	230,000
	48,000	53,000	53,000	—	69,000
	32,000	40,000	30,000	—	31,000
	50,000	47,000	40,000	—	48,000
Average	122,000	118,000	110,000	—	127,000
Butter M.	28,000	30,000	30,000	—	43,000
	24,000	26,000	32,000	—	25,000
	24,700	23,000	26,000	—	29,000
	32,000	32,000	26,000	—	27,700
Average	27,200	27,700	28,500	—	31,200

The multiple counts for each butter represent those of different 1 g. portions (columns). The counts obtained with different media are from the same dilution (rows).

included), is added in sterile solution after the final sterilisation, when the temperature of the medium is about 105° C. The medium is filled out at this temperature and is not subsequently sterilised.

Medium	Initial *p*H	Amount of citric acid monohydrate required per litre for *p*H 3·5 (g.)
Cleared diluted beer wort	*c.* 5·5	2·0
Cleared sweet whey	*c.* 6·5	8·4
Yeast-salt medium	6·6	5·7

Buffer mixture: The addition of 12·8 g. of citric acid monohydrate and 7·2 g. of potassium citrate monohydrate per litre as buffer may be relied upon to adjust the wort and whey media to *p*H 3·5 ± 0·1 (Fig. 4).

The adjustment of media to other pH *values.* Whey, the diluted beer wort and the yeast-salt medium may be adjusted to any *p*H between 3 and 5·5 by reading from the titration curve the amount of citric acid required to bring the medium to the required *p*H.

The ratio of the buffer constituents for this *p*H may similarly be obtained from the buffer curve (Fig. 4).

For *p*H 4·2 (a *p*H which will inhibit most streptococci but not the lactobacilli) add to sweet whey 0·34 per cent. citric acid monohydrate and the buffer mixture in the ratio of 47 parts of citric acid monohydrate to 53 parts of potassium citrate monohydrate.

SUMMARY.

Sources of error in the adjustment of media to *p*H 3·5 have been discussed.

Titration curves for whey and beer wort with citric, lactic and tartaric acids have been constructed. It is shown that citric acid is the most convenient acid to use for adjusting media for yeast and mould counts. The data obtainable from these curves cannot be relied upon to give a medium of *p*H $3{\cdot}5 \pm 0{\cdot}1$, owing to the variations in the composition of whey and wort, but the required accuracy may be obtained by adding to the medium a buffer mixture of citric acid monohydrate (64 parts) and potassium citrate monohydrate (36 parts) in a concentration of 2 per cent. In this way reliable results can be obtained without standardising acids or determining *p*H values. This method may be used for any *p*H between 3 and 6.

An alternative synthetic medium of almost constant composition is described, which may be accurately adjusted by a given amount of citric acid without the addition of the buffer mixture.

ACKNOWLEDGMENTS.

The help of the following is gratefully acknowledged: Messrs Simonds for frequently supplying beer wort, N. Gruber and L. Lawes for technical assistance and Dr A. T. R. Mattick and Mr Wynne-Jones for valuable criticism.

REFERENCES.

(1) HOOD and WHITE (1928). *Can. Dept. Agric.* Pamphlet 92.
(2) MOIR (1931). *Analyst*, **56**, 664, 445.
(3) DAVIS (1931). *Ibid.* **56**, 664, 449.

ABSTRACTS AND REVIEWS

BACTERIOLOGY.

General.

M. O. Robinson. **An aid to more accurate colony counting.** *Amer. J. Pub. Health*, **20**, xi, 1232–3, Nov. 1930.

A specially constructed pen is used which, when pressed against the plate, records the colony electrically, thus saving the worker the fatigue of counting mentally. It is claimed that fatigue and inaccuracy are greatly diminished. J. G. Davis

J. Y. Johnson. **Cultivation of micro-organisms.** B.P. 332, 235, July 11, 1930. (*J. Inst. Brewing*, **36**, xii, 606, December 1930.)

The process described relates to the production of suitable culture media for yeast and other micro-organisms. Peat is hydrolysed by heating with acids, and the resulting liquid, after partial neutralisation, if necessary, is clarified by the addition of metallic compounds, such as sodium silicate, lime, aluminium sulphate, etc., which yield voluminous precipitates. After filtration nutrient salts are added, and the sterile liquid is inoculated with pure cultures of yeast, citromyces or other organisms.

R. Lecoq. **Un milieu vitaminé de préparation simple et rapide pour la culture des microbes.** (Vitamin medium of simple and rapid preparation for the cultivation of microbes.) *C.R. Soc. Biol.* **105**, xxxiii, 636–8, December 5, 1930.

A broth suitable for the cultivation of the commonly occurring bacteria can be quickly prepared from peptone, dried malt extract and certain mineral salts. If a solid medium is required agar-agar may be added. E. R. Hiscox

W. Kopaczewski. **Conditions physico-chimiques de la vitalité microbienne.** (Physical chemical conditions of microbial vitality.) *Archiv f. Mikrobiol.* **2**, ii, 187–244, April 1931.

This paper is an attempt to reduce the factors responsible for biological phenomena such as bacterial antagonism and pathogenicity to physical terms. It contains valuable data for the physical constants of media and cultures of type organisms (*p*H, surface tension, electrical conductivity, viscosity, density and organoleptic properties). J. G. Davis

E. M. Boyd and G. B. Reed. **Gas-metal electrode potentials in sterile culture media for bacteria.** *Canadian J. Res.* **4**, i, 54–68, January 1931.

The potentials obtained with gold, platinum and mercury electrodes in sterile buffered beef broth saturated with air, nitrogen, carbon dioxide and hydrogen are given. It was found that standing the broth anaerobically for a week and then holding the cell at 0° C. increased the constancy of the potential obtained in any one experiment. (Standard deviation, 7 millivolts with platinum, and 22 millivolts with gold electrodes.)

Air caused a slow positive drift at all three electrodes. Hydrogen was without specific effect on gold and mercury, but caused a sharp drop with platinum electrodes. Cessation of the gas current caused a further fall before the return to the initial value. Stirring appeared to cause a negative drift irrespective of the nature of the gas.

Carbon dioxide and nitrogen were found to be inert. Anaerobically aged broths were used in all these experiments and had attained a potential of the order of − 0·1 v. This treatment is therefore equivalent to the de-aeration of broth by nitrogen. In every case the gas metal potentials were found to be in agreement with those reported by French and Kahlenberg for potassium chloride solutions.

Mercury was found to be unreliable and apparently reacted with the medium. The authors suggest that culture potentials may be interpreted by the pressure of gas films on the electrodes. J. G. Davis

H. J. Fuchs. **Eine neue Schnellzentrifuge.** (A new rapid centrifuge.) *Z. Immun. Forsch.* **69**, i–ii, 180–6, 1930.

A type of centrifuge capable of reaching 14,000 revolutions per minute is described. This speed is made possible by reducing the air resistance to the revolving parts; in addition the usual heat production is largely avoided. J. G. Davis

C. D. Cummings. **The use of acid fuchsin in Russell's triple sugar medium.** *J. Inf. Diseases*, **47**, iv, 359–66, October 1930.

Undecolorised acid fuchsin is recommended for the preparation of Andrade's indicator for use in differentiating the coli-typhosus group. The faint pink of the finished medium permits the detection of alkalinity. (The disodium salt (red) is converted by the alkali to the trisodium salt (colourless).)

The Russell medium (*J. Med. Research*, **25**, 217) was found to give correct reactions for *E. typhosus*, *Sal. paratyphosus* A and B, *E. dysenteriae* and *Esch. coli* when undecolorised acid fuchsin was used as the indicator. J. G. Davis

C. S. Mudge. **A life cycle of a thermophilic organism.** *Proc. Soc. Exp. Biol. Med.* **28**, ii, 202–3, November 1930.

One characteristic apparent in the study of thermophilic organisms in pasteurised milk is the sharp rather than gradual increase in bacterial count, a generation time of from 5 to 8 minutes being shown. The conclusion is arrived at that in milk showing thermophilic growth, spores are abundantly present. The life cycle of the organism studied consisted of (1) an invisible spore, abundantly present, heat resistant, incapable of growth on laboratory media at 37° and 60° C.; (2) a vegetative cell (rod) resulting from the germination of (1), destroyed by heat, growing on laboratory media; (3) a spore forming in the rod (2), heat resistant; (4) a spore, heat resistant, non-stainable, incapable of growth on ordinary media; (5) a dormant spore, the dormancy being broken by heat, cold, acids and alkalis. Change from stage (5) to (1) starts the cycle over again. Modifications of the cycles are possible. W. L. Davies

E. W. Todd. **Virulence of haemolytic streptococci. II. The influence of oxygen on the maintenance of virulence in broth cultures. III. The influence of oxygen on the restoration of virulence to matt attenuated cultures.** *Brit. J. Exp. Path.* **11**, vi, 469–79, 480–8, December 1930.

II. This paper describes the investigations of matt virulent and the attenuated (avirulent) variants. The latter may be obtained by anaerobic subcultivation or by the action of small quantities of peroxide over short periods. They cannot be reverted to the virulent form by cultivation in aerated catalose broth. They retain their type-specific substance however, and regain virulence by cultivation in normal serum.

Virulence is maintained by cultivation at high oxygen tension, especially if peroxide accumulation is prevented by catalase.

III. It has been shown that virulence is lost anaerobically but maintained aerobically if peroxide formation is prevented. These findings are discussed in relation to the oxidation-reduction potentials attained by cultures of streptococci.

It is suggested that the ability of serum to restore virulence is due to its oxygen-carrying effect. The increase in virulence is dependent on the oxygen tension of the serum, the optimum being just below that point at which peroxide is formed.

J. G. Davis

G. Gorbach. **Zur Kenntnis der Bakterienproteasen.** (Bacterial proteases.) *Archiv f. Mikrobiol.* **1**, iv, 537–76, November 1930.

A useful summary of previous work on proteases is followed by an account of a detailed investigation of the proteolytic phenomena of six strains of *B. pyocyaneus.*

The enzymes are most readily formed in weakly acid, or neutral solution, and are produced on synthetic media. The optimal concentration of peptone is 0·2–0·4 per cent. and of gelatine 0·5–0·8 per cent. Maximum growth is reached before maximum enzyme activity. The pigment formation goes parallel with that of the enzyme. Optimum conditions for enzyme production are also those for pigmentation.

Gelatine, Witte peptone, leucyl-glycyl-glycine and leucyl-glycine are easily hydrolysed, the optimum *p*H varying with the substrate from 6·5 to 8·4. The optimum temperature is 40° C., and the rate of proteolysis is proportional to the amount of enzyme for low concentrations. Addition of cyanide appears to increase the scission of the gelatine. Heating to 100° C. for 5 seconds is without effect, and for 20 seconds halves the activity of the enzyme. Dialysis does not decrease the enzymic activity. The thus purified enzyme contains no coagulable albumen and gives a negative Millon and tryptophane reaction.

It is absorbed by kaolin and is readily purified in this way, being removed in acid solution.

J. G. Davis

B. C. J. G. Knight and P. Fildes. **Oxidation-reduction studies in relation to bacterial growth. III. The positive limit of oxidation-reduction potential required for the germination of B. tetani spores in vitro.** *Biochem. J.* **24**, v, 1497–1502, 1930.

The spores of *B. tetani* were subjected to different oxidation-reduction potentials which were maintained constant by the poising method previously described (*Biochem. J.* **24**, 1075).

Germination was completely inhibited at an E_h greater than + 0·11 v. at *p*H 7.

J. G. Davis

L. F. Hewitt. **Oxidation-reduction potentials of Pneumococcus cultures. I.** *Biochem. J.* **24**, v, 1551–6, 1930.

Pneumococcus cultures resemble those of haemolytic streptococci in the potentials induced in media in which they are cultured. Thus both aerobically attain the high potential (E_h + 0·4 to 0·5 v.) corresponding to peroxide formation, and are unable to maintain intensely reducing conditions with an abundant supply of oxygen. The difference between the high levels of these two organisms and those of *staphylococci* and *C. diphtheriae* is attributed to the effect of catalase on peroxide formation in the latter case. The effect of different media is recorded.

J. G. Davis

L. F. Hewitt. **Oxidation-reduction potentials of Pneumococcus cultures. II. Effect of catalase.** *Biochem. J.* **25**, i, 169–76, 1931.

Catalase, if added to pneumococcus cultures, prevents the rise in potential subsequent to the logarithmic phase of growth which is apparently due to peroxide formation. Growth is also increased by the addition of catalase. The system removed by catalase does not account for the differences between aerobes and anaerobes. No differences could be detected between bacterial peroxide and hydrogen peroxide. Killed aerobes appeared to possess a reducing system other than catalase; erythrocytes were found to possess a slight poising action at E_h + 0·3 v.

J. G. Davis

A. VAN RAALTE. **New coli test.** *Chem. Weekblad*, **27**, 663, 1930. (*Analyst*, **56**, 660, 199, March 1931.)

In examining milk, 1 c.c. is added to 9 c.c. of medium containing 10 g. each of peptone and sodium chloride and 250 g. of sodium carbonate per litre. Successive dilutions are then made from 1 c.c. of mixture and 9 c.c. of medium so as to give a range containing one part milk in 10, 100, etc., to 100,000. These are tested for indole after 24 hours at 37° C. No reaction should be obtained for a dilution of 1 in 10,000 of ordinary trade milk, or 1 in 100 of "model" or pasteurised milk. Carelessly prepared milk may give a positive reaction in dilutions of 1 in 100,000. The 1 in 10 dilution usually gives negative results owing to coagulation.

G. S. WILSON. **The effect of the virulence of Bact. aertrycke of cultivation in atmospheres containing varying proportions of oxygen.** *J. Hyg.* **30**, iv, 433–67, November 1930.

The virulence of *B. aertrycke* as affected by oxygen tension has been studied by growing a strain under known oxygen tensions. Sub-cultures were made daily and continued until about 90 had been made. Anaerobic cultures maintained a virulence similar to that of the control strain, oxygen tensions from 1 to 21 per cent. caused an increasing decrease and tensions above 40 per cent. produced a culture of increased virulence.

The author reviews the results of other workers and suggests that incubation under anaerobic conditions and in 80 to 100 per cent. oxygen has a stabilising effect on micro-organisms, whereas normal aerobic pressures have the reverse effect.

It is concluded that aerobic cultivation favours microbic dissociation and leads to the replacement of the initial virulent organisms by avirulent variants, and that the agent responsible is present after the stage of active growth has ceased. The addition of serum or blood sometimes favours the reversion of rough avirulent to smooth virulent organisms. Two of the hypotheses are put forward to explain the stabilising effect of anaerobic and hyperaerobic conditions: (1) the agents responsible for dissociation are not formed anaerobically and are oxidised by the high alkalinity and oxygen tension hyperaerobically, (2) virulence is determined by the intracellular *p*H which tends to become alkaline under ordinary aerobic conditions.

J. G. DAVIS

N. UYEI. **The nature of the growth-promoting active principle in the potato in the cultivation of bacteria and especially of the tubercle bacillus.** *Amer. Rev. Tuber.* **22**, ii, 203–17, August 1930.

Potato exerts a marked stimulating effect on the growth of the tubercle bacillus. The effect is not due to the proteins or the salts of the potato.

Glycogen and tyrosine were quite inactive, but maltose, dextrose and inositol stimulated growth if a large inoculum were added, while starch and dextrin exerted a marked stimulation with small inocula. The latter, however, were not so potent as the potato itself.

(The author's distinction between metabolic stimulants (dextrose) and reproductive stimulants (starch) does not appear to be justified in the evidence presented in this paper. The results could be explained equally well by considering the sugars to be specific carbohydrate foodstuffs, and the effect of starch and dextrin due to the presence of traces of bacterial stimulants from which colloids are separated only with difficulty (cf. *Biochem. J.* **23**, 61). Thus although potato starch was active the starchy fraction prepared from autoclaved potato by extraction with hot trichloracetic acid and precipitation with acetone was inactive. The "fractionative properties" of the factor are similar to those of other bacterial factors.) J. G. DAVIS

R. Stenhouse Williams and W. A. Hoy. **The viability of B. tuberculosis (bovinus) on pasture land, in stored faeces and in liquid manure.** *J. Hyg.* **30**, iv, 413–19, November 1930.

The viability of *B. tuberculosis (bovinus)* in exposed faeces is governed mainly by the faeces causing disintegration of the faecal patches. Thus the periods over which survival was proved in faecal patches on pasture land were 5, 2, 2 and 4 months in winter, spring, summer and autumn respectively. In faeces stored in jars in a cool dark cellar virulent organisms were found after 12 months in those naturally infected and after 24 months (probably more) in artificially infected faeces.

In liquid manure stored underground, a gradual decrease in virulence was observed after 6 weeks although virulent bacteria were found after 4 months.

Six pigs were usually used for each test (twelve injections). Comparative tests with antiformin and Petroff's method showed that the former sometimes gave negative results from material shown to contain virulent bacteria by the latter method, a modification of which was therefore used. J. G. Davis

E. Löwenstein. **Technique of pure culture of tubercle bacilli from blood.** *Deuts. med. Wschr.* No. **24**, 1930.

By replacing peptone with asparagin in an egg medium and using the sulphuric acid method for the isolation of the tubercle bacilli from the blood of tubercular patients a much greater percentage of successes occurred. E. C. G. Maddock

R. Stodtmeister. **Die Brauchbarkeit des Harnstoffverfahrens nach Dold zur Anreicherung von Tuberkelbazillen für den mikroskopischen und kulturellen Nachweis.** (Use of Dold's urea enrichment method for microscopic and cultural detection of tubercle bacilli.) *Zbl. f. Bakt.* I, Orig. **118**, iii–iv, 218–29, September 1930.

The author found that the development of tubercle bacilli was less affected by urea than sulphuric acid especially if washed before culture. Washing was not necessary, but better results were obtained with washing. If not washed, cultures grew best on glycerine potato. Tubercle bacilli are not sensitive to high osmotic pressure if not prolonged, but are so if the pressure is suddenly decreased.

E. C. G. Maddock

G. S. Wilson. **The gaseous requirements of Br. abortus (bovine type).** *Brit. J. Exp. Path.* **12**, ii, 88–92, April 1931.

Br. abortus (bovine) does not grow anaerobically. Contrary to the opinion of Bang a reduced oxygen tension does not enhance growth, and increased oxygen tensions do not permit growth in the absence of CO_2. Both oxygen and CO_2 are necessary, the optimal concentrations being 20 and 5–10 per cent. respectively.

It is suggested that CO_2 acts as a specific cell-wall penetrant which raises the intracellular acidity. Other acids cannot replace it. J. G. Davis

A. Harden and M. G. Macfarlane. **Note on the reactivation of reductase in washed yeast preparations.** *Biochem. J.* **25**, iii, 818–21, 1931.

The removal of co-enzyme by washing may best be tested by the failure of the preparation to ferment hexosediphosphate. Addition of lactate, succinate or methylglyoxal to such a preparation restores the ability to reduce methylene blue. Hence it is concluded that a co-enzyme is not required for the action of the reductase.

J. G. Davis

Dairy Bacteriology.

D. R. Wood. **A simple calculation of the limit of value of the microscopical examination of milk for tubercle bacilli.** *Analyst*, **56**, 660, 179–80, March 1931.

By deduction from mathematical consideration of the conditions of examining deposits for tubercle bacilli microscopically it is claimed that the value of this method

is limited to the detection of from 10 to 500 bacilli per c.c. or, excluding cases of bulky deposits which are generally characteristic of tubercular mastitis (*i.e.* deposits of 2·5 c.c. or less from 100 c.c. milk), from 10 to 50 bacilli per c.c. This is 500 times less sensitive than the biological method. W. L. Davies

A. Lumière and A. Dubois. **The distribution of Koch's bacilli, contained in milk, after separating the butter and casein.** Paper at Acad. Sci. (Paris), January 5, 1931. (*Nature*, p. 389, March 7, 1931.)

Known amounts of tubercle bacillus were added to milk, the cream separated, and the casein separated from the butter milk in the usual way. The resulting whey and cream were free from bacilli, which appear to be concentrated in the casein. It is concluded that starting with tuberculous milk the maximum danger is in the cheese.

D. R. Wood and E. T. Illing. **Investigation of the occurrence of B. abortus (Bang) in the milk of English herds (county of Somerset).** *Analyst*, **56**, 659, 105, February 1931.

Milk (100 c.c.) from each of 379 Somerset herds was centrifuged and the deposit injected subcutaneously into a guinea pig, which was killed after 4 weeks and culture from the interior of the spleen made on glucose, neutral red vitamin agar in 10 per cent. carbon dioxide at 37° C. *B. coli* was usually not found. *Br. abortus* was found in the milk of four herds and identified by agglutination with standard anti-serum. J. G. Davis

J. H. Brown. **Experiments on the shipment of milk samples for bacteriological examination.** *Certified Milk*, pp. 220–1, 1929.

Samples of milk when dried in small vacuum tubes and stored for some days yield approximately the same number and kinds of bacteria that were contained in the original milk.

Attempts to absorb milk in silica gel for delayed bacteriological analysis failed, as although the same types could be recovered the numbers were much diminished. J. G. Davis

H. N. Heffernan. **The most probable numbers of B. coli per 100 c.c. of milk.** *Certified Milk*, pp. 73–5, 1929.

This paper is a report on the results obtained from examining 451 samples of certified milk for the most probable number of coli. This figure fell from 125–240 in 1928 to 2–4 per 100 c.c. (1929). High coli was associated with high counts in all but two cases.

A modified McCrady table was used for the calculation of the results. (*J. Infect. Dis.* **21**, 287, 1917.) J. G. Davis

J. H. Brown. **The detection of Bacillus coli and related organisms in certified milk.** *Certified Milk*, pp. 220–38, 1929.

This paper describes the attempts to detect *B. coli* (added artificially) in certified milk by plating and by fermentation tube methods. It was found that when few coliform organisms were present (1 to 10 per 0·1 c.c. as shown by Chalmers' taurocholate agar), gas sometimes failed to appear in the fermentation tubes. The Chalmers' medium is recommended for low count milk and it is suggested that plating in this medium followed by the fishing of suspected colonies on to Simmon's citrate agar would be a simple and reliable method for the determination of *B. coli* and *B. lactis aerogenes* in certified milk. J. G. Davis

R. J. Ramsay and P. H. Tracy. **Food poisoning probably caused by orange-coloured staphylococcus from udders of apparently healthy cows.** *Proc. Soc. Exp. Biol. Med.* **28**, iv, 390–1, January 1931.

While studying the cause of malt flavour in raw milk one of the authors became ill with severe gastro-enteritis, showing symptoms of food poisoning. The suspected food was raw milk inoculated with a pure culture of an orange-coloured staphylococcus. Four kittens fed with the inoculated milk also contracted severe symptoms of food poisoning. Other cases of food poisoning have been traced to a similar type of toxin-producing staphylococcus. *Staph. aureus* produces a malt-like flavour in milk and the authors believe that the incriminated organism is identical with it.

W. L. Davies

H. Alfonsus and O. Correll. **Nachweis von thermophilen Bakterien mittels der Reduktaseprobe bei 63 Gr. Cels. und ihre praktische Anwendung im Molkereibetrieb.** (Detection of thermophilic bacteria by the reductase test at 63° C. and its practical use in the dairy industry.) *Molkereiztg.* **1**, 1237–9, 1930. (*Zbl. f. d. ges. Hyg.* **23**, iii, 146, October 10, 1930.)

The methylene-blue reductase test at 63° C. is recommended as a simple method for detecting the presence of thermophilic organisms in milk. (This has also been found by Meanwell who followed the growth of thermophilic organisms in a pasteurising plant by this method, the standard plate count being useless for the purpose.)

Milk produced and handled under clean conditions does not contain large numbers of thermophilic bacteria.

J. G. Davis

C. Gorini. **Acid proteolytic bacteria in pasteurised milk.** *Compt. rend.* **191**, 885–6, 1930. (*Chem. Abst.* **25**, vi, 1291, March 20, 1931.)

The thermophilic, acid-proteolytic bacilli of the subtilus group are among the most harmful to the preservation of pasteurised milk. The preservation of milk after pasteurisation depends more upon the number of bacteria which have resisted the heat treatment than upon the types present.

M. Grimes, V. C. E. Kennelly and H. A. Cummins. **A study of fungi found in butter.** *Sci. Proc. Roy. Dublin Soc.* **19** (N.S.), xlvii, 549–69, October 1930.

A brief description is given of various fungi isolated from sweet cream butter during an investigation of the causes of deterioration of such butter. The resistance of these fungi, to high temperatures, their salt tolerance and their growth at low temperatures are recorded.

E. R. Hiscox

G. F. V. Morgan. **Some colour-producing organisms in butter-washing water.** *New Zealand J. Agric.* **41**, v, 315–8, November 1930.

An account of certain cultural characteristics and the influence on butter of four pigment-producing organisms isolated from the water supplies of dairy factories.

E. R. Hiscox

Boisseau and Mesnil. **Rôle de la Monilia dans la fermentation du Pont-l'Évêque.** (Rôle of Monilia in the fermentation of Pont-l'Évêque cheese.) *C.R. Acad. Agric. France*, **15**, 580–3, 1929. (*Biol. Abst.* **4**, xi, 2746, November 1930.)

The active agent in ripening is a Monilia. Growth of the mycelium throughout the cheese slowly breaks down the casein to NH_3 and prevents growth of *Oidium lactis* and other moulds which would ripen the cheese too rapidly and would result in off flavours. Adequate growth of Monilia is assured by using milk 22–3° acidity (Dornic) or of 0·22–0·23 per cent. lactic acid and by making the cheese in a warm room to aid acid production and draining off the cheese. Active growth of monilia, effective salting, acid curd, and proper temperature conditions are indispensable.

W. Stocker. **Ueber bakterielle Schwarzfärbungen des Käserinde.** (Bacterial black discoloration in cheese rind.) *Zbl. f. Bakt.* II, **82**, xv–xxii, 405–6, December 1930.

The occurrence of black spots on the rind of soft cheese usually accompanied by a darkening of the whole surface and extending to a few mm. below the surface, was usually traced to the presence of *Monilia nigra*. In some cases, however, the mould was absent and the discoloration appeared to be due to bacteria which were capable of inducing a characteristic blackening in milk. There was no coagulation of the milk but complete digestion took place. The optimum temperature for the formation of the pigment was 12° C. and the colour was intensified by an alkaline reaction.

E. R. Hiscox

G. Koestler. **Ueber die von der Rinde her sich vollziehende Entsäuerung des Hartkäseteiges.** (Complete deacidification of the curd of hard cheese from the rind inwards.) *Land. Jahrb. d. Schweiz*, pp. 1065–76, 1929.

By measurements of the hydrogen-ion concentration at different depths throughout the ripening, the author procures evidence to support Freudenreich's theory that hard cheese ripen uniformly throughout the mass. In addition, however, the experiments show that in cheese such as Emmental and Greuerz there is, side by side with the principal ripening processes, a gradual deacidification of the curd from the rind inwards due to the action of micro-organisms. L. A. Allen

CHEMISTRY (ORGANIC, INORGANIC AND PHYSICAL).

General.

T. B. Brighton and C. M. Dice. **Increasing the purity of common salt.** *Ind. Eng. Chem.* **23**, iii, 336–9, March 1931.

Substances causing an odour when refined common salt is dissolved in hot water are present in such minute amounts that their identification is not possible. Heating fine salt in an oxidising atmosphere above 210° C. eradicates the odour. Since such heating causes discoloration of salt it is advisable to wash with brine before roasting. This treatment is possible on a commercial scale resulting in a whiter and purer dairy and table salt. W. L. Davies

R. Cohen. **Permeability of packing materials (cellophane, etc.) to water and water vapour.** *Chem. Weekblad.* **28**, 159–62, 1931.

On comparing the permeabilities of cellophane, duratex with that of parchment, the data obtained was so irregular that general conclusions only could be drawn. Permeability to moisture is inversely related to electrical insulating power both being connected with the presence or absence of free hydroxyl groups. W. L. Davies

R. J. Best. **The application of the quinhydrone electrode to the measurement of the acid reaction of unbuffered solutions.** *J. Phys. Chem.* **34**, viii, 1815–17, August 1930.

Values given by the quinhydrone electrode in unbuffered solutions (standard HCl of varying strengths) gave good agreement with theoretical values for *p*H 1 to *p*H 5. At values above this unreliable results were obtained. (The *p*H of saturated aqueous quinhydrone is about 5·85.) Equilibrium was established in ½–1½ minutes. Electrodes only washed and then immersed in another solution drifted to an equilibrium value. J. G. Davis

J. L. R. Morgan, O. M. Lammert and M. A. Campbell. **The quinhydrone electrode. I.** *J. Amer. Chem. Soc.* **53**, ii, 454–69, February 1931.

The reproducibility of the quinhydrone electrode under different conditions of electrode size, age and composition has been studied. Generally, reproducibility with ease and precision is attainable but the greatest source of error lies in the preparation of the metal electrode. A method for the preparation of a robust, serviceable electrode is given. Cracks in the glass cause relatively large errors and results obtained from alcohol flaming of the electrode do not justify risks following on the formation of minute cracks. Electrodes of long wire or of foil, which need not be of a greater dimension than 1 cm.^{2}, give greater precision than short wires, which generally give erratic results. The composition and age of the metal is of little importance although old roughened platinum foil (less than 1 cm.^{2}) gave the best results in 0·1 *N* hydrochloric acid. W. L. Davies

O. M. Lammert, J. L. R. Morgan and M. A. Campbell. **The quinhydrone electrode. II.** *J. Amer. Chem. Soc.* **53**, ii, 597–604, February 1931.

In investigations of the effect of nitrogen on the reproducibility of the quinhydrone electrode (in 0·1*N* HCl) it was found that more reproducible results were given by either drying the electrodes in nitrogen or preparing with solutions stirred with nitrogen than by electrodes prepared in air or oxygen. Saturating the liquid of the half-cell with nitrogen also gave more consistent results. In the presence of nitrogen, equilibrium potential was reached more quickly and short circuiting pairs of electrodes had no effect on the P.D. obtained in nitrogen. The authors claim that the 0·1 *N* HCl quinhydrone half-cell prepared in this way is the most easily and quickly reproducible electrode which they have used. W. L. Davies

E. H. Lepper and C. J. Martin. **On the behaviour of "indifferent" electrodes when used for the determination of oxidation-reduction potentials in the presence of hydrogen.** *Biochem. J.* **25**, i, 45–8, 1931.

The behaviour of gold and iridium electrodes in poised and unpoised buffer solutions and in the presence of nitrogen, oxygen and hydrogen has been examined.

Hydrogen is much more readily adsorbed by the iridium electrode which in the presence of this element tends to function as a hydrogen electrode. 0·002 *M* indigo carmine "poised" the iridium electrode, but when the concentration was reduced to 0·0001 *M* the electrode attained the potential of the hydrogen electrode in half an hour. J. G. Davis

A. Taffel and C. Revis. **The determination of the rancidity in oils and fats.** *J. Soc. Chem. Ind.* **50**, xi, 87–91 T, March 13, 1931.

Oils, air-blown or allowed to become rancid at various temperatures, contain a proportion of easily reducible peroxides (by hydriodic acid) and some other form of oxygen less easily reducible. This latter form of oxygen increases in proportion with the temperature of blowing. Oils blown at 170° C. contain mostly the less easily reducible oxides. Methods are described to evaluate the amount of each type of oxide present in an oil.

A modified Kreis test is described which is claimed to give more accurate comparative results. Intensity of colour with the Kreis reaction followed closely the peroxide content of oils.

The two modified tests are advantageous in that they can be universally applied to any type of rancid oil with definite and reproducible results and in being able to distinguish oils which have been blown at high temperatures from those which have grown rancid normally. W. L. Davies

A. F. McCarley. **Some new colour reactions with certain oils and fats.** *Analyst*, **56**, 659, 104–5, February 1931.

Certain phenols give well-marked colour reactions in chloroform solution with certain oils and fats in the presence of bromine in excess. Three drops of oil are added to a saturated solution of resorcinol in chloroform and five drops of strongly brominated hydrochloric acid added. A colour gradually forms on shaking. Various oils including cod-liver oil and butterfat give varying shades of pink. Probably colour development is associated with Vitamin A content since the unsaponifiable matter gives the colour reactions and the separated fatty acids do not. Cod-liver oil gives the most pronounced coloration, followed by butterfat, which is much less reactive.

W. L. Davies

D. P. Grettie and R. C. Newton. **Measurement of rate of formation of oxidative decomposition products in fats and oils.** *Ind. and Eng. Chem.* (anal. ed.), **3**, ii, 171–3, April 1931.

2 g. of fat are soaked into a pleated filter paper sheet which is inserted into a tube passing through an air oven maintained at 100° C. A slow stream of air (1 c.c. per second) is drawn through the tube and bubbled through a solution of 0·01 *N* permanganate acidified with sulphuric acid kept at 25° C. in a water bath. The volatile products of fat oxidation reduce the permanganate, the amount of reduction being determined during suitable periods of time (20 minutes). It is found that the odour of rancidity developed when the volatile products are sufficient to reduce 1 c.c. of 0·01 *N* permanganate in 20 minutes.

W. L. Davies

J. Grossfeld and F. Battay. **Versuche über Nachweis, Bestimmung und Vorkommen der Buttersäure in Lebensmitteln.** (Detection, determination and occurrence of butyric acid in foodstuffs.) *Z. f. Unters. Lebensm.* **61**, ii, 129–61, February 1931.

One part of butyric acid in 12,500 is detectable from its odour by distilling the aqueous solution after removal of other substances by oxidation with permanganate. Details are given for the method of distillation (phosphoric acid), oxidation of other volatile substances and extraction of higher fatty acids. A method for the analysis of the resulting liquid for acetic and butyric acids is given. Milk was found to contain 0·004 per cent. (sweet) and 0·008 per cent. (sour), while cheese varied in content of free butyric acid from 0·066 to 1·340 per cent. according to the type of cheese.

W. L. Davies

E. O. Whittier and S. P. Gould. **Speed of crystallisation of lactose, galactose, glucose and sucrose from pure solution.** *Ind. and Eng. Chem.* **23**, vi, 670–2, June 1931.

In the earlier stages of crystallisation of lactose, the rate of crystallisation of the isomeric form separating is the principal factor. Later, the rate is decreased, and the rate of attainment of equilibrium between isomeric forms becomes the controlling factor. The most rapid crystallisation of lactose occurs from a solution maintained at 30° C. for 3 hours which is then allowed to cool to 20° C.

W. L. Davies

H. P. Morris, J. W. Nelson and L. S. Palmer. **Quantitative determination of calcium, magnesium and phosphorus in feedstuffs and cattle excreta.** *Ind. and Eng. Chem.* (anal. ed.), **3**, ii, 164–7, April 1931.

With materials containing large quantities of substances insoluble in hydrochloric acid, such as hay and faeces, the determinations of calcium and magnesium tend to give low results owing to the occurrence of these metals as complex silicates. These silicates are broken down by fusion with sodium carbonate in platinum vessels.

Silicic acid does not interfere with the volumetric estimation of calcium, but the dehydration method of removing silicic acid must be resorted to in the gravimetric estimation. W. L. DAVIES

W. J. WILEY. **The dissociation of calcium citrate.** *Biochem. J.* **24**, iv, 856–9, 1930.

Measurements were made of the conductivity of calcium citrate solutions of different dilutions, and confirmation obtained by conductivity titrations of citric acid solutions with calcium hydroxide solutions. The small values obtained for the degree of dissociation of tricalcium citrate in dilute aqueous solutions are recorded. S. J. ROWLAND

DAIRY CHEMISTRY.

G. D. ELSDON and J. R. STUBBS. **The freezing-point of milk and its applications.** *J. Soc. Chem. Ind.* **50**, xvi, 135–41T, April 17, 1931.

A review is given of the apparatus used for the determination of the freezing-point of milk, with a detailed description of the Hortvet apparatus and method, together with the results obtained by various workers. The depression of freezing-point obtained by the authors for a large number of genuine milk samples ranged from 0·533° to 0·559°, with an average of 0·543°. The various corrections for acidity applied by different workers are discussed and it is suggested that, owing to ignorance of the acidity of the fresh sample of an unknown milk examined after further acidity has developed, only fresh samples of milk should be worked on. When acidity is reckoned as ml. 0·1 *N* soda per 10 ml. of milk, and when the acidity of a milk sample exceeds 2·5, a rough value of the freezing-point of the original milk can be obtained by subtracting 0·003 from the observed depression for every 0·1 ml. over 2·0 ml. of 0·1 *N* soda.

The value of the test is discussed and examples are given of the detection of added water by this method. The average depression for genuine milk may be taken as 0·54°, but no sample should be considered as watered on the evidence of a single sample unless this falls below 0·53°. W. L. DAVIES

G. W. MONIER-WILLIAMS. **The nitrate test for the detection of added water in milk.** *Analyst*, **56**, 663, 397–8, June 1931.

The blue colour formed in this test is due to the quinone-imonium salt of diphenylbenzidine. Some of the nitrate is used for the oxidation of some diphenylamine to diphenylbenzidine and the second oxidation forms the blue colour. If only a trace of nitrate is present in the milk, it may happen that no nitrate is left to carry out the second oxidation. Hence, it is suggested that diphenylbenzidine should be used to give a more sensitive reaction. Details of the method of preparation of diphenylbenzidine from diphenylamine are given. W. L. DAVIES

G. JØRGENSEN. **The rising of fat in milk.** *Analyst*, **56**, 663, 380–2, June 1931.

This is a criticism of Stock's findings (*Analyst*, **55**, 535, 1930) on the same question. When milk is allowed to stand in a churn the percentage decrease in the fat content of the lower layer of milk is greatest in the first hour of standing, but the rate of decrease depends on the diameter of the container, the narrower the container the less the decrease of fat content. Samples of milk show considerable variation in velocity of fat rising. The possibility of an increase of the solids-not-fat content of the cream layer by adsorption, evaporation and oxidation is discussed.

W. L. DAVIES

K. Sarlo. **Determination of the sodium bicarbonate added to milk.** *Chem. Ztg.* **55**, 374–5, 1931. (*Brit. Chem. Abst.* B, p. 651, July 10, 1931.)

The solid residue obtained by evaporating 10 c.c. of milk is carefully ashed, taken up with H_2O, and treated in the presence of a few grams of Zn with a few c.c. of 20 per cent. HCl, in a special apparatus. The mixture of CO_2 and H is bubbled slowly through 22 c.c. of saturated CaO water of known concentration for 30–35 minutes. The residual $Ca(OH)_2$ is then titrated with 0·04 *N* H_2SO_4 against methyl-orange. Normal milk ash contains 1·5–2·0 per cent. CO_2 and a deduction of 1·8 c.c. from the titre is made in evaluating the amount of added bicarbonate, which is then given by the formula $NaHCO_3$ (mg./l.) = 336 × (22 – 1·1 v. – 1·80), where v. is the volume of H_2SO_4 required. If the factor 1·6 is used instead of 336, the "neutralisation" of the milk in degrees acidity is given. The method is sensitive to within 1° acidity and is suitable for routine testing.

W. Mohr and C. Brockman. **Oberflächenspannungsmessungen an Milch.** (Surface tension measurements of milk.) *Milchw. Forsch.* **10**, i–ii, 72–95, May 1930.

The authors have measured the surface tension of milk both by a "torsion" and by a "drop" method. Although the results obtained by both methods usually run parallel, those of the "drop" method are consistently higher.

The authors suggest that membrane formation is the cause of anomalous results which are obtained when determining the surface tension of skim milk.

Different methods of treatment such as low temperature pasteurisation, high temperature pasteurisation, repeated boiling and sterilisation are entirely without influence on the surface tension of milk, as also are ageing, freezing and the addition of formalin.

An increased air content causes a rise in the surface tension. With increasing acidity the surface tension diminishes, as the calcium caseinate is split up.

E. C. V. Mattick

G. Koestler. **Weitere Untersuchungen ueber Ziegenmilch.** (Recent research on goat's milk.) 1930. Liebefeld, Bern.

This paper reports the studies of the composition of numerous samples of goat's milk (and comparison of it with cow's) which were carried out in the hope of elucidating the anaemia which occurs in animals fed on it. Goat's milk had been found to be adequate for growth of rats on a vitamin free ration and equivalent to cow's. It is constantly higher in chlorine content than cow's milk and the low bacterial counts of the milk show that such a difference as this is due to normal secretory activity of the gland and not to disease. Other differences noted in goat's milk were (1) lower lactose concentration, (2) lower in nitrogenous constituents, (3) lower in citric acid, (4) notably low cholesterol content, and (5) less tendency to lactic fermentation, with very delayed clotting. During the colostrol period goat's milk is closely analogous to cow's. The fat of goat's milk contains odoriferous matter, probably a mixture of volatile fatty acids (especially caprytic and caproic). G. L. Peskett

C. H. Whitnah. **Indications of glucose in milk.** *J. Amer. Chem. Soc.* **53**, i, 300–4, January 1931.

Fermentation of milk with washed bakers' yeast was found to remove added glucose completely without acting on lactose. Applying this method for the detection of glucose occurring naturally in milk, it was found that in 275 samples examined, a decrease in specific rotation after yeast treatment gave glucose contents varying from zero to 0·35 per cent. Eleven other samples showed slight increases in rotation after fermentation. W. L. Davies

H. T. Gebhardt and H. H. Sommer. **Determination of copper in dairy products.** *Ind. Eng. Chem.* (anal. ed.), **3**, i, 24–6, January 1931.

A modified Biazzo method for the colorimetric determination of small traces of copper in dairy products is described. Ashing of milk at temperatures above 565° C. was found to cause loss of copper, and it is recommended not to exceed that temperature in the ashing process. The precipitated copper sulphide is collected in a chloroform layer instead of by filtering, the chloroform being finally boiled off. The subsequent procedure follows that of the Elvehjem-Lindow method. Testing the method by adding known amounts of copper to milk and recovering gave satisfactory results.
W. L. Davies

L. H. Lampitt and M. Bogod. **The determination of lactic acid in milk and milk products.** *C.R. IX Cong. Chim. Indust., Chim. et Ind., Spec. No.* 510–5, March 1930. (*Chem. Abst.* **24**, xxi, 5386, November 10, 1930.)

The method depends on the oxidation of lactic acid to acetaldehyde by means of potassium permanganate. To 10 g. of milk, 4 g. of condensed milk, or 1 g. dried milk, are added 10 ml. of 0·01 *N* NaOH and 30 ml. of water. After boiling and cooling, the liquid is transferred into a 100 ml. flask 10 ml. of 0·1 *N* H_2SO_4 added and 5 ml. of phosphotungstic acid reagent (saturated solution in *N* hydrochloric acid), cooled and made up to the mark. Twenty ml. of a filtrate from this are transferred to a 50 ml. flask, 2 ml. of saturated copper sulphate solution and 3 g. of $Ca(OH)_2$ are added, and made up to the mark. After standing for 20 minutes the contents are centrifuged (2000–2500 r.p.m.) for 5 minutes, the clear liquid filtered. Of the filtrate 25 ml. are transferred to a 250 ml. distillation flask containing a little talc and 10 ml. water; 1 ml. of 5 *N* H_2SO_4 are added and the flask attached to a condenser. A dropping funnel containing 100 ml. of 0·004 *N* $KMnO_4$ is fitted. An adapter at the end of the condenser fits into a flask (100–110 ml.) containing 10 ml. of 0·02 *N* (approx.) sodium bisulphite solution, the tip being below the level of the liquid. On heating the flask, as soon as boiling begins, the permanganate solution is allowed to drop on to the boiling liquid; boiling is carried on for 50–60 minutes, the dropping being so regulated as also to take this time (80–100 ml. distillate). The excess of bisulphite in the receiver is titrated with 0·01 *N* iodine and the bisulphite in 10 ml. of the original bisulphite solution also determined. The difference in the titrations multiplied by 0·45 gives the amount of lactic acid in mg. Loss of lactic acid during the clarifying processes amounts to about 10 per cent., so that a truer value of the lactic acid is obtained by multiplying the found value by 10/9. Determinations carried out on milk show that the total lactic acid content of normal milk is less than the equivalent of the titratable acidity. This difference diminishes or changes its sign if added alkali is present. W. L. Davies

A. Staffe. **Sur quelques composants et propriétés du lait des vaches castrées.** (Some components and properties of the milk of castrated cows.) *Le Lait*, **10**, c, 1087–96, December 1930.

W. H. E. Reid. **Effect of pasteurisation temperature on the physical properties of milk.** *Missouri Agric. Exp. Sta. Res. Bull.* **126**, 1929. (*Brit. Chem. Abst.* B, p. 1128, December 12, 1930.)

The viscosity, specific gravity and surface tension are not materially affected by the use of different pasteurisation temperatures; rise of the temperature retards the separation of fat.

C. K. Johns. **Modification of the methylene-blue reductase test and its comparative value in determining the keeping quality of milk.** *Sci. Agric.* **11**, 171–90, 1930. (*Brit. Chem. Abst.* B, p. 271, March 20, 1931.)

The modifications ensure greater convenience and accuracy of working (especially with high-grade milks) and closer correlation with the keeping quality. A preliminary incubation at 12·8° for 18 hours is adopted, and in the subsequent incubation at blood heat tubes not discoloured after 6 hours are shaken to redistribute bacteria carried to the surface with the rising butterfat.

K. Yamamura. **Effect of the heating temperature of milk and estimation of the heating temperature of dairy products.** *J. Agric. Chem. Soc. Japan*, **6**, 462–73, 1930. (*Chem. Abst.* **24**, xxi, 5387, November 10, 1930.)

A. E. Perkins. **Relation between the quantities of fat and protein in normal milk.** *Ohio Agric. Exp. Sta. Bull.* No. **446**, 126–7, 1930. (*Brit. Chem. Abst.* B, p. 271, March 20, 1931.)

From analyses of grouped samples of milk the following formula is derived: protein (per cent.) = 2·78 + [0·42 (fat per cent. − 2·78)] ± 0·25. The probable error of single determinations from mixed milk is likely to be smaller than that shown above.

G. N. Quam. **The temperature effect on the solubilities of nickel, tin, copper, steel (chromium) and zinc in raw cow milk.** *Proc. Iowa Acad. Sci.* **36**, 261–2 (1929). (*Chem. Abst.* **25**, vi, 1291, March 20, 1931.)

The solubility curves for Ni, Cu and Zn show a maximum within a range of 75° to 85°. Sn shows an accumulation of sturdy corrosion product above 75°, while the Cr steel (super-ascoloy) shows no apparent change.

D. R. Wood, E. T. Illing and A. E. Fletcher. **The diphenylamine test for nitrates in milk as a means of detecting added water and the effect of drenching cows with "nitre."** *Analyst*, **56**, 661, 248–9, April 1931.

Lerrigo's test (*Analyst*, **55**, 433, 1930) was used for this investigation and found to be satisfactory. No samples of genuine milk gave tests for nitrates. Five per cent. added water could be detected if the water contained 0·5 parts per 100,000 of nitrate nitrogen. Cows drenched with $\frac{1}{3}$ oz. of "nitre" daily did not cause any nitrate to be secreted in milk. Attention is drawn to filter paper sometimes containing sufficient nitrate to cause fictitious results. W. L. Davies

G. E. Holm and B. H. Webb. **Buffer capacities of various milks and proprietary infant foods.** *Amer. J. Dis. Child.* **40**, 260–8, 1930. (*Physiol. Abst.* **15**, x, 587, January 1931.)

Any correlation observed between buffer capacities and types of curd formed is probably accidental, and in infant feeding the nature of the curds formed is of greater importance than is the buffer capacity.

A. Schneck and H. Mengebier. **Ueber die Extinktion der Milch.** (Opacity of milk as measured by the extinction coefficient.) *Milchw. Forsch.* **11**, i–ii, 1–29, October 1930.

Under strictly controlled conditions light transmission in milk is proportional to the concentration of the constituents, the Lambert-Beerscke law for solutions applying. The fat phase has little effect. Dilutions of milk from 1 to 100 also obey the Lambert-Beerscke law. Skimming depressed the extinction by 4 per cent. in many of the samples tested. Milk samples from individual cows differ in extinction coefficients. The small effect due to fat varies with the average size of the fat globules. W. L. Davies

MINISTRY OF HEALTH. **Sale of Food and Drugs Act.** Extract from 1929–30 Annual Report and Reports of Public Analysts 1929. (*Analyst*, **56**, 659, 110–11, February 1931.)

Milk. Of 68,115 samples examined, 5293 (7·8 per cent.) were reported against (previous year 8·2 per cent.). Outstanding cases were deficiencies in fat of 43·3 and 10·3 per cent. Dirt was present in 22 samples, annatto in 10, and 5 samples contained preservatives. There were 13 cases of added water to separated or skimmed milk. Out of 226 samples of dried milk, 3 were reported against and out of 1294 samples of condensed milk 41 were deficient, mostly in fat.

Cream. Out of 51 adulterated creams (2368 samples) 37 contained boron preservative, 10 tinned creams were low in milk fat or solids. Two samples of "fresh" cream were reconstituted.

Butter. Out of 10,965 samples of butter, 149 were reported against, 81 containing excess water, 49 containing foreign fats and 19 with boron preservative.

Cheese. Of 1321 cheese samples, 27 were adulterated. Generally they did not attain the composition necessitated by their description. No tin-wrapped cheese examined was free from tin contamination. W. L. DAVIES

G. T. PYNE. **The detection of viscogen in cream.** *Analyst*, **55**, 657, 747–9, December 1930.

Viscogen (calcium saccharate) owes its thickening properties mainly to the interaction of its calcium with soluble phosphates in milk and cream whereby gelatinous tricalcic phosphate is formed. The addition of soluble oxalate leads to the formation of calcium oxalate which reverses the thickening effect of the saccharate. The test thus consists of comparing the viscosities of a cream before and after adding potassium oxalate solution. Cream containing viscogen shows a very large drop in viscosity on the addition of oxalate, but cream with no viscogen shows a slight rise in viscosity. Certain precautions as to mixing and adjustment of acidity of the cream after the addition of oxalate have to be observed. W. L. DAVIES

P. H. TRACY and H. A. RUEHE. **Factors affecting the heat coagulation of homogenized coffee cream.** *Illinois Agr. Exp. Sta. Bull.* **352**, 1930.

The following factors are contributory to the feathering of fresh cream in coffee: the presence of calcium salts in the water used for making coffee, and excess of soluble calcium salts in the cream, high fat content of the cream, increasing the viscosity of the cream by homogenisation at low temperature, increasing the fat-adsorbing area in the cream by increased homogenising pressures and using an improper combination of pressures when using the two-stage homogeniser. Feathering may be controlled by using water of low calcium content for coffee making, the addition of sodium bicarbonate, citrate or phosphate to the cream (if legally permissible), pasteurising the cream at 155° F. for 30 minutes and homogenising at 500–1000 lb. pressure for the single-stage machine or, with the two-stage machine, at a total pressure of 1500–2500 with 500–1000 lb. pressure on the second valve. A daily laboratory test on each batch should be carried out. W. L. DAVIES

E. ELSER. **Semi-micro methods for the analysis of condensed milk.** *Mitt. Lebensm. Hyg.* **21**, 314–20 (1930). (*Chem. Abst.* **25**, vi, 1291, March 20, 1931.)

About 8–9 g. of condensed milk is diluted to 200 c.c. with water. For total solids 20 c.c. is evaporated with pumice on the steam bath, then at 100° *in vacuo* for 2 hours. For sugars the proteins in 50 c.c. are peptonised with $CuSO_4$ solution and 20 c.c. of the filtrate is taken for lactose and similarly for sucrose. Total protein and albumin-globulin are determined by the micro-Kjeldahl method. Fat is estimated in 11 c.c. with the Gerber butyrometer. Cl is determined by treating 20 c.c. with concentrated

HNO_3 and 0·02 *N* $AgNO_3$, destroying proteins with perhydrol and titrating back with 0·02 *N* KCNS. Ash is determined on the undiluted product, which is dried first at 70° and then at 120° *in vacuo* before ashing. The ash is dissolved in HNO_3 and used for determination of Ca, K, Fe and P_2O_5.

L. H. Lampitt and J. H. Bushill. **Physico-chemical constitution of spray-dried milk powder. Fat in spray-dried milk powder.** *J. Soc. Chem. Ind.* **50**, vi, 45–54 T, February 6, 1931.

The "free" fat of milk powder, or fat extractable by organic solvents varied from 3·3 to 14·2 in spray-process milk powder, and 91·6 to 95·8 for roller-process powder, the results being calculated on total fat. The extraction process consisted of allowing 2 g. of powder to remain in contact with 100 ml. of a fat solvent (carbon bisulphide) for 18 hours (shaking for the first hour). The solvent was filtered (Green 591½) and the dissolved fat determined by weighing after distilling off the solvent. For high "free" fat powders this value agreed well with that obtained by Soxhlet's method. Grinding the powder increased the amount of "free" fat to 83 per cent. of the total fat for the spray-process powder, and to practically all the fat of the roller-process powder. The reasons for these differences are dealt with. The behaviour of milk powder when absorbing moisture was remarkable, in that it first became clammy, then hard and powdery, this stage corresponding to the "freeing" of the fat, and was called the condition of "critical moisture" content (8·6–9·2 per cent. moisture). Amorphous lactose crystallised at this point. Drying out this absorbed moisture did not cause reversion to the original condition. The critical moisture range was found to be proportional to the solids-not-fat content of the powder. Lactose in its amorphous state thus had a marked effect on the availability of the fat to solvents and crystallisation of the lactose "frees" the fat. W. L. Davies

E. S. Guthrie. **Air in butter.** *J. Dairy Sci.* **13**, vi, 461–70, November 1930.

A method for determining air in butter depending on the fact that air would be the only constituent of butter capable of expanding in a vacuum, has been described. The air content of thoroughly worked butter was higher than that of well-worked butter, although a great variation was encountered between the values for identically treated samples. An average air content (by volume) of well-worked butter was 4·65 per cent. and that of thoroughly worked butter, 5·37 per cent. This variation in air content is of significance in the marketing of butter, where print butter is packed by volume and sold by weight. W. L. Davies

R. Bhattacharya and T. P. Hilditch. **The fatty acids and component glycerides of Indian ghee.** *Analyst*, **56**, 660, 161–70, March 1931.

Two samples each of buffalo and cow ghee have been investigated. The samples of cow ghee, one from a stall-fed, the other from a pasture-fed animal gave figures which lie between the limits of eight English and New Zealand butters already examined. The R.M. values were low and the oleic acid contents were of average value. Stearic acid was higher in the fat of the stall-fed animal. The buffalo ghees were characterised by relatively lower contents of oleic and linoleic acids, but a higher content of butyric acid. Stearic acid was higher, and there were small but definite amounts of arachidic acid present. The glyceride structure of these fats confirmed previous findings in that the esters are of the mixed "heterogeneous" type. The saturated fatty acids were found in each part of the fat-fully saturated and mixed saturated-unsaturated triglycerides. Tripalmitin, tristearin and triolein would be present only in small amounts. W. L. Davies

BIOCHEMISTRY.

T. P. Hilditch and J. J. Sleightholme. **The glyceride structure of butterfats.** *Biochem. J.* **25**, ii, 507–22, 1931.

Analytical data are given for the fatty acids present in the fully saturated glycerides of four English butterfats and one New Zealand butterfat. The component fatty acids of the whole butterfats having been previously determined (Hilditch and Sleightholme, *Biochem. J.* **24**, iv, 1098, 1930), the general features of the glyceride structure of butterfats are discussed, as revealed by these figures taken in conjunction with those formerly obtained for two New Zealand butterfats (Hilditch and Jones, *Analyst*, **54**, 75, 1929). Consideration is given to the general distribution of unsaturated acids throughout the glycerides, and to certain regularities in the occurrence of the saturated acid components in all the fats except the two obtained from cows receiving specific oil-cakes in their diet. S. J. Rowland

I. Pasztor. **Die Veränderung des Wassergehaltes beim Käseschmelzen.** (The change in water content during the processing of cheese.) *Kisérl. Közlemenyek*, **32**, 492, 1929. (*Milchw. Forsch.* **10**, i–ii, Ref. 35, May 1930.)

The water content of Emmental increases during processing on an average by 8·8 per cent., that of Romadur by 5·3 per cent. from that of the original raw cheese.

The moisture content of processed Emmental cheese of good quality lies between 42 and 46 per cent., while that of Romadur lies between 50 and 55 per cent. It depends upon the water content of the raw cheese, the amount of citrate solution which is added, and the period of steam heating in the vacuum. E. C. V. Mattick

G. Wode. **Détermination du *p*H dans le jus de fromage.** (*p*H determination of the press extract of cheese.) *Le Lait*, **10**, c, 1083–7, December 1930.

Time-potential measurements obtained when measuring *p*H values of cheese by the quinhydrone method are discussed. It is shown that the apparent rise in *p*H noticeable within 5 to 10 minutes after the electrode has been in the quinhydrone cheese pressed extract mixture increases with the age of the cheese and is correlated with the appearance of a red colour caused by reaction between the quinhydrone and the amino acids of the cheese. Addition of water to the cheese juice increases the period of stability of the potential and also inhibits the coloration, but slightly raises the *p*H. J. G. Davis

S. Pasztor. **Die Bestimmung der Wasserstoffionekonzentration im Schmelzkäse mit Hilfe der Chinhydronmethode.** (Determination of hydrogen ion concentration in processed cheese by means of the quinhydrone electrode.) *Molkereiztg. Hildesheim*, No. **98**, 1831, 1930. (*Milchw. Forsch.* **11**, i–ii, Ref. p. 27, October 1930.)

Addition of water to cheese raised its *p*H by about 0·04 (1 vol.) up to about 0·60 (99 vols.).

The initial values were about 5·5. J. G. Davis

B. Dyer and G. Taylor. **County of Essex—Report of the County Analyst for the fourth quarter, 1930. Tin in cheese.** *Analyst*, **56**, 661, 251–3, April 1931.

Of 35 samples examined during the quarter none was entirely free from tin, the variation being 0·3 to 5·5 grains per pound (43 to 786 p.p.m.), the higher figure being an exceptional maximum.

Based on the investigations of the Ministry of Health in 1908 on the tin contamination of "canned" foods the authors suggest that contamination up to 2 grains per pound (286 p.p.m.) need not be taken too seriously, whereas anything above

this arbitrary figure should be regarded as excessive. Twenty-eight (or 80 per cent.) of the samples did not exceed this limit. These included prepared cheese from home-made and Dominion Cheddars and a large proportion of continental origin (Gruyère). The seven samples containing tin above the arbitrary limit were Swiss or French Gruyère. It is suggested that packers should pay more attention to quality of foil used and to the shortening of the interval between the packing and consumption.

W. L. DAVIES

D. C. CARPENTER. **The molecular weight of casein. III.** *J. Amer. Chem. Soc.* **53**, v, 1812–26, May 1931.

Previous work has shown the molecular weight of casein to lie between 75,000 and 100,000. Analyses of casein for various elements and groupings have given the following figures: sulphur, 0·785 per cent.; phosphorus, 0·856 per cent.; cystine, 0·488 per cent.; tryptophane, 1·237 per cent.; tyrosine, 5·55 per cent.; histidine, 1·776 per cent. Multiples of the lowest molecular weight needed to contain one atom or one molecular residue of the above list agree with extraordinary accuracy to give a molecular weight ranging from 97,796 to 98,988, an average of 98,000 being taken as the probable value. A molecule of casein of this molecular weight contains 24 sulphur and 27 phosphorus atoms, 2 cystine, 6 tryptophane, 30 tyrosine, and 11 histidine molecules in its constitution.

W. L. DAVIES

G. M. MOIR, **Determination of the milk proteins. I. The chemistry of the separation of casein. II. The identity of the casein precipitate. III. Proposed modified method for casein. IV. A. The combined determination of albumin and globulin. B. The separate determination of albumin and globulin.** *Analyst*, **56**, 2–9; 73–8; 147–9; 228–35; January–April 1931.

Casein. The chemistry of casein and the state in which it exists in milk have been discussed in order to show the desirability of using an acid reagent for the purpose of its analytical separation. Experiments have been carried out which show that by mixing definite quantities of milk and a suitable acetic acid-sodium acetate buffer solution, a *p*H close to the isoelectric point of casein can be obtained with cows' milk of widely varying composition. Even when the milk has soured somewhat, the effect of this on the final *p*H of the mixture is slight. Experiments have shown that in order to precipitate a maximum of casein from milk it is desirable to add the buffer solution in two parts, acid first, and then sodium acetate. When this is done the maximum casein values for milk are obtained between *p*H 4·5–4·7.

Upon the fundamental basis of the conception of the isoelectric point, it has been suggested that casein be defined as the material which is precipitated from cows' milk at *p*H 4·6, by acetic acid buffered by sodium acetate. Two methods have been used to show that casein precipitated in this way is chemically identical with the material precipitated at *p*H 4·2 by acetic acid alone. (*a*) When the two caseins are digested with normal caustic soda at 37° C., the production of amino groups as determined by the increase of the formol titration takes place at the same rate. (*b*) The rate of oxidation of the two caseins by sodium hypobromite *under comparable conditions* is the same for both.

Details of the proposed new method are as follows: Into a weighed covered beaker (100–150 ml.) pipette 10 ml. of the well mixed sample, and weigh again quickly. Dilute with at least 50 ml. of water which has first been warmed to 40–42° C. Add at once 1·5 ml. of 1·67 *N* (10 per cent.) acetic acid, and then stir very gently. After allowing to stand about 20 minutes, add 4·5 ml. of 0·25 *N* sodium acetate solution, and after stirring gently leave for at least an hour. Filter through a 9 cm. No. 42 Whatman filter (fluted folding), and at once wash the precipitate three times by decantation. During two further washings break up the precipitate and transfer to

the paper, and finally rinse the rim of the paper with a fine jet of water. Without delay clean out the beaker two or three times by adding water and successive portions of the sulphuric acid required for the Kjeldahl digestion, taking care to remove all the casein from the lip of the beaker and the stirring rod. Sundry explanatory notes are added and directions given (Paper 1) for treating milks of other animals besides the cow. The result should be expressed as per cent. of casein *nitrogen*.

Albumin and globulin. A study of previous work, supplemented by the writer's experiments, show that for the *combined* determination of these proteins the heat-coagulation method gives quite low results, while those obtained by the use of tannic acid are slightly high. Consequently the following method is tentatively proposed. To the filtrate obtained after the isoelectric precipitation of the casein sufficient trichloroacetic acid is added to make the final concentration approximately 4 per cent. The mixture is heated for half an hour on the boiling water bath, and after standing to cool, it is filtered and washed with a 1 per cent. solution of trichloroacetic acid. The nitrogen content of the precipitate is estimated by Kjeldahl's method. The *separate* determination of albumin and globulin is a very difficult problem which ought not to be attempted without consulting the literature cited in this paper.

G. M. Moir

P. Arup. **The analysis and composition of vegetable parchment used for packing dairy products.** *Analyst*, **56**, 660, 149–61, March 1931.

Forty-one samples of vegetable parchment and two of grease-proof paper were analysed for moisture, ash, water-soluble material and reducing material. Bursting strengths, wet and dry, and ability to support mould growth were also investigated. Tentative standards are suggested, namely: moisture, maximum, 10 per cent.; ash, 0·45 per cent.; water soluble extract, 1·3 per cent.; bursting strength, minimum, 25 lb. per square inch, for parchments of 25 lb. per ream and 18 lb. per square inch. for 18 lb. per ream. The strength after wetting should be at least a third of the dry strengths. The percentages of reducing material are given as a guide to the detection of added sugar. The reducing material was found to consist of degradation products of ligno-cellulose.

W. L. Davies

O. Warburg, F. Kubowitz and W. Christian. **Ueber die katalytische Wirkung von Methylenblau in lebenden Zellen.** (Catalytic action of methylene blue in living cells.) *Biochem. Z.* **227**, iv–vi, 245–71, October 1930.

Methylene blue increases the oxygen consumption of blood cells by 20–30 times. Methhaemoglobin is first formed and is then reduced to haemoglobin quantitatively by the carbohydrate present. The methylene blue catalyses the oxidation of ferrous iron to ferric, which then catalyses the oxidation of the carbohydrate. In the absence of carbohydrate, methhaemoglobin accumulates.

J. G. Davis

M. A. B. Fixsen. **The biological values of proteins. II. The biological value of purified caseinogen, and the influence of vitamin B_2 upon biological values, determined by the balance sheet method.** *Biochem. J.* **24**, iv, 1794–1804, 1930.

The biological value of purified caseinogen was redetermined by metabolism experiments on adult rats, and found to be 45, a figure considerably lower than that of previous works. The technique was improved by supplying the B vitamins as concentrates containing only small amounts of nitrogen.

The absence of either vitamin B_1 or B_2 caused a decline of appetite within 48 hours; this was so great in the absence of B_1 that the intake of calories was too low for reliable figures to be obtained for the calculation of biological values.

The absence of vitamin B_2 did not appear to prevent the economical use of ingested nitrogen, provided the calorie intake from fat and carbohydrate was adequate.

S. J. Rowland

E. J. Quinn, J. G. Hartley and M. A. Derow. **Some observations on the behaviour of vitamin A in or from primary sources.** *J. Biol. Chem.* **89**, ii, 657–63, December 1930.

The vitamin A in dry plant tissue is susceptible to destruction during storage. 70 per cent. loss in vitamin A occurred in dry spinach during 15 months storage. Addition of rancid fat to dry plant tissue caused a greater amount of destruction than in the dry material alone. Vegetable oils readily dissolve the vitamin from various types of plant tissue. Ultra-violet irradiation of petroleum ether extracts of carrot readily destroys vitamin A derived from this primary source. W. L. Davies

E. M. Hume and H. Henderson Smith. **The value of foodstuffs for vitamin A.** *Lancet*, 1362–3, December 20, 1930.

The authors have estimated the vitamin A content of several foodstuffs using as a criterion the maintenance of rats depleted of their vitamin A reserves, over a set experimental period. They found that the minimal daily rat dose of spinach is 20 mg. or less, of cabbage more than 20 but less than 80 mg., of New Zealand butter 28 mg. or less. The corresponding dose of carotene is 0·003 mg. It is suggested that the evaluation of foodstuffs might be standardised against the value of a standard preparation of carotene. S. K. Kon

H. A. Mattill. **Anti-oxidants and the auto-oxidation of fats.** *J. Biol. Chem.* **90**, i, 141–51, January 1931.

This work was carried out to secure information as to the chemical nature of the anti-oxygenic substances found present in natural oils which prevent the auto-oxidative destruction of fat-soluble vitamins. The anti-oxygenic capacity of phenols resides in two hydroxyl groups being either in the ortho or para positions; when they are in the meta position the compound is inactive. The hydroxyl groups must also be directly attached to the nucleus. α-naphthol is more effective than β-naphthol; while α-naphthoquinone is inactive. Sterols of animal origin and sitosterol from plant sources were inactive. It is suggested that the existence of pro- and anti-oxygenic substances among the unsaponifiable constituents of natural fats may be concerned with the physiological action of the fat soluble vitamins. W. L. Davies

M. J. Cummings and H. A. Mattill. **The auto-oxidation of fats with reference to their destructive effects on vitamin E.** *J. Nutrition*, **3**, iv, 421–32, January 1931.

The auto-oxidisability of the fats, cod-liver oil, lard, butterfat, cottonseed oil, stearin, and hydrogenated cottonseed oil, was determined by measuring the induction period of oxygen absorption, and this susceptibility to oxidation was correlated with the reproductive behaviour of rats, fed on food mixtures containing these fats. The mixture of butter and hydrogenated cottonseed oil was most satisfactory while the mixtures, lard, cod-liver oil, and stearin butter were least successful. The efficiency of a given source of vitamin E depends in part on the auto-oxidisable materials and the anti-oxygens associated with it. The part played by vitamin E itself as an anti-oxygen is discussed. W. L. Davies

B. Sure. **Dietary requirements for fertility and lactation. I. The role of fat soluble vitamins in fertility and lactation.** *Arkansas Agr. Exp. Sta. Bull.* **250**, June 1930.

In this and the following two bulletins the author assembles and summarises his published researches, begun in 1919, which have appeared in scientific periodicals. In the first bulletin lack of fertility or significant success in rearing of young on skimmed milk powder rations is discussed and is attributed to a dietary factor other than protein, the fat soluble vitamin A, the antirachitic vitamin or the water-soluble vitamin. Evidence is presented showing that the ethereal extracts of yellow maize,

wheat embryo, and hemp seed contain a hitherto unrecognised organic factor which is essential for reproduction. The factor was also present in commercial cottonseed oil and commercial olive oil, but absent from commercial coconut, linseed or sesame oils. The requirement of vitamin B for normal mammary gland function is much greater than that for growth. The name vitamin E has been suggested by the author for the reproductive factor. Vitamin E deficiency in the female albino rat is characterised by resorption of the foetus during gestation. The hypothesis is advanced that vitamin E may be composed of two fat soluble vitamins one essential for fertility, and another indispensable for lactation. Cod-liver oil as employed in the laboratory of the author has been demonstrated to be deficient in vitamin E. When administered in excessive amounts to animals receiving a purified synthetic diet containing 5 per cent. Harris yeast, the same oil had a toxic effect on growing animals especially after mating. Butterfat was a much less potent source of vitamin E than wheat germ oil, but when introduced to the extent of 10 per cent. in a synthetic diet causing sterility it assured not only continuous fertility but also a noticeable success in lactation.

Vitamin B is one of the limiting factors, from the point of view of lactation, of various skimmed milk powder and whole milk powder diets studied.

Sterility characterised by resorption of the foetus during gestation and associated with vitamin A deficiency has been produced in rats on skimmed milk powder diet containing an abundance of vitamin E. Later work does not lend support to the dual theory of vitamin E previously advanced by the author, vitamin E must be considered exclusively as an antisterility vitamin. At the end of the bulletin the historical aspect of the discovery of vitamin E and the work of other investigators are discussed. S. K. Kon

B. Sure. **Dietary requirements for fertility and lactation. II. The role of vitamin B in lactation and vitamin requirements of nursing young.** *Arkansas Agr. Exp. Sta. Bull.* **251,** June 1930.

A cold 75 per cent. ethyl alcoholic extract of 6·5 g. of whole wheat embryo per animal per day furnished enough vitamin B for excellent growth. It was necessary, however, to supply the same alcoholic extract of at least 22·4 g. of wheat germ per lactating rat per day to furnish sufficient vitamin B for normal lactation. A cold 25 per cent. ethyl alcoholic extract of approximately 13·0 g. of the same wheat embryo per nursing animal per day furnished sufficient vitamin B for normal rearing and weaning of young.

The requirements of vitamin B for normal mammary gland function were found considerably greater than that for optimum growth.

A quantitive biological method for the study of vitamin B requirement for normal lactation has been described. By such a method it became apparent that yeasts from various sources vary considerably in their biological value as a source of vitamin B for lactation. It was found that a concentrated preparation from yeast, prepared by releasing vitamin B from a yeast extract adsorbed on fuller's earth with sodium hydroxide, was three times as potent for normal lactation as the Harris yeast secured from the Harris Laboratories, Tuckahoe, New York.

By removing the daily yeast dosage (as a source of vitamin B) from the lactating mother during the latter part of lactation, when the nursing young are failing or when they show prolonged maintenance, rearing of the young was expedited and infant mortality at the same time circumvented. Upon applying the same technique with yeast concentrates, it was discovered that the lactating mother dissipates at least 60 per cent., if not more, of the vitamin B dosage daily apportioned to it in the metabolism of transfer to the milk.

To avoid infant mortality, nursing young need large amounts even of highly concentrated products of vitamin B daily during lactation.

The large requirements of vitamin B for lactation can now be explained as due to the inability of the nursing mother to secrete vitamin B quantitatively in the milk.

Accelerated growth of nursing young can be obtained by administration of liberal quantities of potent concentrated preparation of vitamin B.

A quantitative biological method has been perfected for the vitamin B requirements of nursing young of the albino rat, which method is sensitive to the extent of 1 mg. per nursling per day.

A vitamin B unit, applied to nursing young, has been defined as follows: the vitamin B unit is the daily amount of vitamin B which must become available to a nursing young of the albino rat, weighing 30 g., in order to permit a gain of 10 g. in 7 to 10 days.

1 g. of a dehydrated baker's yeast "Federal" brand, has been found to contain 7 units of vitamin B. By applying the quantitative biological method of assay to vitamin B concentrates, variations were found to the extent that 1 g. contained as little as 14·4 units, and as much as 125 units.

Nursing young of the albino rat need approximately 100 times as much of the vitamin B complex (calculated in terms of dehydrated baker's yeast), as vitamins A and D (as furnished by a brand of cod-liver oil employed in the laboratory since 1921) for continuous growth during the nursing period.

Copper was found to have no supplementary value to a vitamin B concentrate for lactation.

A differentiation was made of the vitamin B complex in rice polishings as evidenced in studies of lactation.

The lactating rat is unable to secrete quantitatively in the milk the antineuritic as well as the antipellagric factor, both of which are essential for growth.

Rice polishings contain, in addition to large amounts of the antineuritic factor for lactation, appreciable amounts of the antipellagric factor for growth.

Biological technique has been described for the production of uncomplicated vitamin B deficiency in nursing young of the albino rat. AUTHOR'S SUMMARY

B. SURE. **Dietary requirements for fertility and lactation. III. Pathological changes in nursing young of the albino rat suffering from vitamin B deficiency, and the role of vitamin B in infant nutrition.** *Arkansas Agr. Exp. Sta. Bull.* **252**, June 1930.

Nursing young of the albino rat suffering from a deficiency of vitamin B complex were found to die with accompanying hemorrhages in the osteogenetic tissues. Hemorrhages were also found in certain of the viscera.

Polyneuritic nursing young were found usually to die with their stomachs well distended with curd. The diets on which beriberi or polyneuritis were produced in the nursing young proved entirely satisfactory for maternal welfare and for the growth of non-lactating individuals.

Nursing young of the albino rat suffering from uncomplicated vitamin B deficiency develop marked hypoglycemia at a stage of the avitaminosis before loss of body weight has occurred. In such avitaminosis nursing young also develop anhydremia and marked disturbance in hematopoietic function. Vitamin therapy produces a rapid increase in concentration of blood sugar, and a regeneration of blood in polyneuritic nursing young of the albino rat.

Nursing young of the albino rat suffering from a deficiency of the vitamin B complex develop anhydremia, as is evidenced from hemoglobin determinations, erythrocyte counts, determinations of total blood solids, and studies of the refractive index of the blood serum. Such young also show marked disturbances in the hematopoietic function.

The lactating rat, whose nursing young are suffering from uncomplicated vitamin B deficiency, shows a normal range of concentration of alkaline reserve of the blood.

Uncomplicated vitamin B deficiency in nursing young of the albino rat, during the stage of prolonged maintenance and incipient polyneuritis, has no influence on the concentration of alkaline reserve of the blood. A reduction in concentration is noted only in the terminal stages of the disease, indicating acidosis.

The most noteworthy biochemical change in nursing young of the albino rat suffering from uncomplicated vitamin B deficiency is the marked reduction in the glycogen content of the liver.

A summary of the role of vitamin B in infant nutrition has been presented.

AUTHOR'S SUMMARY

H. O. SMITH and V. E. NELSON. **Cod-liver oil for reproduction and lactation.** *Proc. Soc. Exp. Biol. Med.* **28**, iv, 393, January 1931.

Various samples of cod-liver oil were tested for their potency in the reproductive vitamin by introducing them into a standard ration and assessing the potency on the number of young produced per female per month with a "growing" ration as control. The oils were found to vary in vitamin E content and to contain less than some natural foods. Ability to support the young was the basis of assessing the lactation-encouraging potency and mortality of the young varied with the kind of oil and the level of feeding. Mortality of pregnant females bore no relation to the potency of the oil in vitamin E.

W. L. DAVIES

L. J. PEET, P. M. NELSON and E. H. SMITH. **Meat in nutrition. II. Some dietary factors influencing lactation.** *J. Nutrition*, **3**, iii, 313–23, November 1930.

Reproduction and lactation responses in albino rats fed on a balanced ration containing pressure-cooked lean beef as the main source of protein, were observed through three generations. Dietary modifications were added during the latter part of pregnancy and throughout lactation. Mothers on increased meat diet plus 15 per cent. yeast weaned the largest percentages of young in the three generations; but for the first two generations mothers on basal diet with autoclaved yeast addition reared an even larger percentage. Growth response in the young was greatest for the increased meat plus yeast diet. Increasing the meat protein of the diet tended to delay the onset of pregnancy.

W. L. DAVIES

E. WATCHORN. **Irradiated ergosterol and calcium-free diet: effect on calcium and phosphorus metabolism.** *Biochem. J.* **24**, v, 1560–3, 1930.

Calcium and phosphorus retention in rats on Steenbock's rachitogenic diet (2965) modified to contain 49 mg. of calcium and 60 mg. of phosphorus per 10 g. of diet is decreased by excessive doses (0·05 per cent. of the diet) of irradiated ergosterol. The rats lost weight rapidly. On a similar diet, but containing 121·7 mg. phosphorus for 10 g. of food and only traces of calcium, the same level of irradiated ergosterol does not effect the phosphorus metabolism, but causes elimination of calcium by the kidney. The calcium is probably lost from the bones or tissues. The animals continued to gain weight.

S. K. KON

E. WATCHORN. **The absorption and excretion of calcium and phosphorus by rats receiving excessive doses of irradiated ergosterol.** *Biochem. J.* **24**, iii, 631–40, 1930.

Calcium and phosphorus retention in rats is decreased by excessive doses of irradiated ergosterol fed in addition to a synthetic diet of caseinogen, rice, starch, arachis oil and salt mixture. The urinary calcium is greatly increased but not the phosphorus. The faecal content of both calcium and phosphorus decreases but not always in proportion to the intake. Even greater disturbances in retention and absorption occur during the recovery period after the administration of the irradiated

ergosterol has been discontinued. The low food intake and loss of weight characteristic of the hyper-vitaminosis probably contribute to some of these results. The deposits of calcium found in certain tissues as the results of large doses of irradiated ergosterol cannot be accounted for on the grounds of increased absorption and retention. They are probably derived from the bones. S. K. KON

B. SJOLLEMA and L. SEEKLES. **Ueber Störungen des mineralen Regulationsmechanismus bei Krankheiten des Rindes (Ein Beitrag zur Tetaniefrage).** (Impairment of the mineral regulatory mechanism in diseases of cattle (A contribution to the problem of tetany).) *Biochem. Z.* **229**, iv–vi, 358–80, December 1930.

Magnesium, ionised Ca, diffusible Ca, total calcium and inorganic phosphorus were estimated in the blood serum and in the ultra-filtrate from blood serum of normal cattle and of cows suffering from milk fever and grass staggers. The following average figures were obtained:

		Blood serum Mg./100 c.c.					Ultra filtrate Mg./100 c.c.		
	Aver. of	Ca	Mg	P	Aver. of	Diff. Ca	Ca++	Mg	P
Normal	12	9·35	1·66	4·57	12	5·23	1·65	1·24	4·6
Milk fever	25	4·35	2·19	2·16	10	2·36	0·44	—	—
Grass staggers	55	6·65	0·455	4·33	18	4·19	1·09	0·27	4·10

The authors are inclined to link up the decrease of the Ca/Mg ration in milk fever (2) and increase in grass staggers (14·6) as compared with the normal (5·6) with corresponding change in excitability accompanying the two diseases.

In grass staggers the serum magnesium was always low at the onset of a tetanic seizure but relatively high afterwards. The percentage of diffusible calcium was much more variable in milk fever and grass staggers than under normal conditions.

It is believed that a toxin either present in, or formed from the fodder is responsible for grass staggers. S. K. KON

M. G. BERTRAND and Y. BRANDT-BEAUZEMONT. **Sur la teneur en zinc du foie chez le rat en voie de croissance.** (Zinc content of the liver of the growing rat.) *Ann. Inst. Pasteur*, **46**, v, 572–3, May 1931.

Previous investigations of the zinc content of animals at various stages of growth have been repeated on the livers of rats. Values obtained at the age of 1, 15, 30 and 210 days were, 114, 51, 37 and 34 mg. per cent. of dry weight, thus confirming previous work on whole animals which showed a similar fall, due to the low zinc content of milk.

The behaviour of this element resembles that of iron as shown by Bunge.

J. G. DAVIS

H. J. WARTH and N. K. AYYAR. **The influence of foodstuffs on the acid-base balance of cattle urines.** *Biochem. J.* **24**, iv, 1595–1600, 1930.

This paper describes a method for the determination of the acid—base balance of the cattle urine, based on a potentiometric titration of the urine freed from CO_2 and figures for chlorides, sulphates, ammonia and hippuric acid.

Details are given of the results obtained at Bangalore by the application of this method to urines from green fodders, over ripe grasses and cereal straws, covering several varieties for each of these types. Marked differences in composition and reaction are recorded. S. J. ROWLAND

M. STEUBER and A. SEIFERT. **Lactose in the economics of the growing organism.** *Arch. Kinderheilk.* **85**, 12–23, 1928. (*Chem. Abst.* **24**, xx, 5057, October 20, 1930.)

A synthesis of fat from lactose, on a fat-free diet was not demonstrated. In the infant, lactose is not a suitable biological substitute for fat.

S. S. McCosh, I. G. Macy and H. A. Hunscher. **Human milk studies. VI. Vitamin potency as influenced by supplementing the maternal diet with yeast.** *J. Biol. Chem.* **90**, i, 1–13, January 1931.

The vitamin B content of the breast milk from three women was determined by rat experiments, before and after supplementing the usual maternal diets with 10 g. yeast daily. The vitamin B content was found to be inversely proportional to the volume of milk secreted daily. The yeast-supplemented diet transferred into the milk a substance capable of making the experimental animals utilise their food more economically without augmenting food consumption. W. L. Davies

W. E. Kraus and F. C. Mourve. **A comparison of the influence of iodised milk and of potassium iodide administered directly, on the size and iodine content of the thyroid gland of rats.** *J. Biol. Chem.* **89**, ii, 581–8, December 1930.

The iodine content of milk may vary from a trace up to several parts per million depending on regional influences, and feeding cows with iodised mineral mixtures tends to make milk contain more than its normal content of iodine. Such "iodised milk" and samples of milk to which equivalent amounts of iodine as potassium iodide were added, on comparison with normal milk fed to white rats, were found to produce smaller thyroid glands of higher iodine content. For therapeutic and prophylactic purposes it is considered that direct addition of iodine to milk is more economical and controllable than the feeding of iodine-containing rations to cows. A short discussion on the advantages and disadvantages of an iodised milk supply is included.
W. L. Davies

S. J. Erler and K. J. Pawlowsky. **Der Stickstoffwechsel beim Saugkalb mit Milchfütterung.** (The nitrogen metabolism of milk fed calves.) *Biochem. Z.* **228**, i–iii, 89–100, November 1930.

The different forms of nitrogenous excretory products found in the urine of suckling calves varied but slightly from day to day. The urine was alkaline, containing but a trace of uric acid, a constant amount of creatine (creatine coefficient 20·33) and a considerable amount of hippuric acid. The ammonia content was low. The urea nitrogen decreased with age for calves of the same breed. W. L. Davies

D. L. Drabkin and H. K. Miller. **Haemoglobin production. II. The relief of anaemia, due to milk diet, by feeding amino acids.** *J. Biol. Chem.* **90**, ii, 531–43, February 1931.

Milk anaemia in rats was relieved by the addition of amino acids to milk containing an insufficient quantity of iron. The amino acids, arginine, glutamic acid and their salts proved effective for haemoglobin regeneration. Tryptophane, pyrrolidonecarboxylic acid, sodium aspartate and a proline mixture produced an initial increase in haemoglobin and remained at the higher level except in the case of sodium aspartate where severe anaemia again set in. Alanine and histidine and their salts produced no relief. With increase in haemoglobin, growth steadily took place after the stunting effect of anaemia. W. L. Davies

R. Stenhouse Williams and E. C. V. Mattick. **The importance of a complete study of the nutritional value of milk.** *J. State Med.* **39**, iii, 141–56, March 1931.

This is the report of a lecture given at the Royal Institute of Public Health. The authors dealt particularly with some of the known effects of heating milk upon the vitamin content, the proteins, ferments and salts, and with the results of feeding experiments which had been carried out upon the nutritional value of milk, when fed to school children and babies. They pointed out the necessity for further work along these lines, particularly for determining whether the nutritive value of milk was impaired by heating. E. C. V. Mattick

R. Hess. **The treatment of rickets with irradiated milk.** *Strahlentherapie*, **34**, 787–97. (*Chem. Abst.* **24**, xxii, 5806, November 20, 1930.)

Hess reports the results of experiments with milk irradiated with the Scheidt apparatus or with the Hanau quartz lamp in CO_2. Ultracina or Vigantol were used for comparative purposes. Röntgen pictures and clinical findings served as the criteria. Fresh milk irradiated with the Scheidt apparatus gave positive results in about 30 per cent. of the cases; considerably more favourable results were obtained with the Hanau apparatus. The reasons for this uncertainty are shown. The treatment of rickets with irradiated milk is believed to deserve further attention. More favourable results are expected with an improved apparatus.

G. Leighton and P. L. McKinlay. **Milk consumption and the growth of school children. Report on an investigation in Lanarkshire Schools.** Edinburgh: H.M.S.O. 1930, pp. 20.

This report gives the results of an extensive 4 months' experiment carried out in Lanarkshire schools to test the influence of the addition of ¾ pint of milk daily to the diet of children between the ages of 5 and 12 years. 5000 children received raw Grade A (T.T.) milk, 5000 received similar milk after pasteurisation, and 10,000 children were measured as controls.

The authors conclude that the milk produced a definite increase in growth, but that they found nothing to support the belief that younger children derived more benefit than older ones. Also that raw and pasteurised milk was so far as they could judge, equal in growth promotion.

Some of the results are shown in the following tables:

Average increase in weights (in ounces) in the three groups.

	Boys			Girls		
Age	Control	Raw milk	Pasteurised milk	Control	Raw milk	Pasteurised milk
5–	11·64	14·88	15·65	7·00	14·50	6·62
6–	13·75	13·51	9·96	11·21	10·61	10·05
7–	11·17	14·85	15·55	8·90	11·22	12·94
8–	11·38	14·21	15·21	9·77	13·40	13·37
9–	9·53	13·43	11·83	7·87	13·81	12·52
10–	7·10	13·53	10·39	9·51	15·08	18·96
11–	6·14	12·74	11·05	12·62	24·92	17·08
Average of all ages	10·06	13·78	12·42	9·74	14·41	13·82

Average increase in heights (in inches) in three groups.

	Boys			Girls		
Age	Control	Raw milk	Pasteurised milk	Control	Raw milk	Pasteurised milk
5–	0·75	0·95	0·94	0·86	0·64	0·87
6–	0·80	0·87	0·87	0·80	0·86	0·84
7–	0·76	0·87	0·82	0·75	0·84	0·81
8–	0·74	0·82	0·79	0·71	0·81	0·78
9–	0·69	0·80	0·74	0·66	0·76	0·78
10–	0·68	0·76	0·68	0·71	0·79	0·72
11–	0·69	0·74	0·70	0·77	0·86	0·81
Average of all ages	0·728	0·814	0·772	0·730	0·814	0·790

Differences of changes of weight and height in raw and pasteurised milk-fed groups.*

Age	Weight		Height	
	Boys	Girls	Boys	Girls
5–	−0·77 ±3·19	7·88 ±4·02	0·01 ±0·075	−0·23 ±0·055
6–	3·55 ±0·94	0·56 ±0·94	0·00 ±0·018	0·02 ±0·015
7–	−0·70 ±1·08	−1·72 ±1·04	0·05 ±0·019	0·03 ±0·019
8–	−1·00 ±1·00	0·03 ±1·00	0·03 ±0·018	0·03 ±0·017
9–	1·60 ±0·98	1·29 ±1·12	0·06 ±0·017	−0·02 ±0·016
10–	3·14 ±1·24	−3·88 ±1·28	0·08 ±0·019	0·07 ±0·018
11–	1·69 ±1·44	7·84 ±1·68	0·04 ±0·021	0·05 ±0·026
Average of all ages	1·30	0·42	0·044	0·026

* A negative sign indicates a difference in favour of the pasteurised milk-fed group.

[In criticism of the experiment it may be pointed out that in selecting the children a system of random choice was used but not adhered to, also only one kind of milk was used in one school so that direct comparison between the kinds of milk is less reliable than if both milks were used in all schools. Again while there can be no doubt that the milk diet increased growth very considerably, it is not difficult to show that the conclusion drawn by the authors as to equality in the growth value of raw and pasteurised milk is statistically unsound. In actual fact the results show a significant advantage in favour of raw milk.] S. Bartlett

M. V. K. Nelson. **The growth and nitrogen metabolism of infants receiving undiluted milk.** *Amer. J. Dis. Child.* **39**, 701–10, 1930. (*Physiol. Abst.* **15**, x, 587, January 1931.)

In nine healthy infants the total nitrogen retention, the retention per kilogram, and the percentage of intake retained were greater on undiluted milk than when they received diluted milk and therefore less food. There was an increased rate of growth in both weight and length.

F. Kolbe. **Vigantol und bestrahlte Milch.** (Vigantol and irradiated milk.) *Z. f. Fleisch- u. Milchhyg.* **41**, iii, 47–9, November 1, 1930.

A review with forty-seven references, of the German work on the antirachitic value of irradiated ergosterol and irradiated milk. S. K. Kon

H. Taylor. **Haematopoietic properties of dried milk.** *Lancet*, No. **5593**, 1041, November 8, 1930.

This letter draws attention to the work of Mackay, which had been neglected by Bacharach in his article on the subject. G. L. Peskett

J. E. Becker and E. V. McCollum. **Dietary deficiencies of milk.** *Amer. J. Hyg.* **12**, ii, 503, September 1930.

The authors employed a basal diet consisting of whole milk powder (Klim) and dextrinised starch. By varying the level of dextrinised starch many substances were added to the basal diet. Of 38 diets in which milk powder was used at a 60–85 per cent. level in combination with other substances, 24 sufficed for production of young. In 4 cases fifth generations have been obtained; the supplements in these diets were cooked dried beef liver 4 and 5 per cent. (60 per cent. of whole milk powder), yeast 5 per cent. (60 per cent. dried milk powder) and a combination of ferric citrate (0·5 per cent.) and copper sulphate (0·1 per cent.) (70 per cent. whole milk powder). The authors think that it is the richness of liver in iron and copper that makes it an effective supplement to milk. S. K. Kon

ROEMMELE and STÖHR. **Ueber den Einfluss eines mit d-Vitamin durchsetzten Futters auf die Milch.** (Influence on milk of fodder enriched with vitamin D.) *Milchw. Forsch.* **10**, v–vi, 413–23, October 1930.

100 g. of maize flour containing 1 c.c. of a solution of vitamin D (potency not stated) in oil were added daily for 6 weeks to the normal diet of four milch cows. This amount was increased to 1000 g. daily (containing 10 c.c. of the solution of vitamin D) for a subsequent period of 8 days. Four control cows received during similar periods equal amounts of maize flour from which the vitamin D solution was omitted. The milk from the two groups was compared and *no* difference was found in the vitamin D content, the percentage of fat, the calcium content, the milk yield, the melting point of the fat and in the suitability for cheese making.

[Steenbock, Hart, Hanning and Humphrey (*J. Biol. Chem.* **88**, i, 197–213, August 1930) have recently shown that the antirachitic potency of milk can be increased by feeding milch cows 50 g. of irradiated yeast daily (equivalent to 50,000 Steenbock rat units of vitamin D), but that the milk yield and fat content of the milk are not affected. It is difficult to appraise the value of the negative findings of Roemmele and Stöhr concerning the vitamin D content of the milk from their experimental cows, as the antirachitic potency of the preparation fed is not stated.] S. K. KON

W. SCHEIMPFLUG. **Vergleichende Untersuchungen über antirachitische Wirkung von nach Scholl und nach Hoffmann bestrahlter Milch an Ratten.** (Comparative investigations on the antirachitic action on rats of milk irradiated by the Scholl and Hoffmann methods.) *Milchw. Forsch.* **10**, v–vi, 455–80, October 1930.

Rats rendered rachitic by feeding the rachitogenic diet of Pappenheim, McKann and Zucker were cured by the daily administration of 1 c.c. of milk irradiated with ultra-violet light by the method of Scholl. Milk irradiated by the Hoffmann method was ineffective at the same level and was undistinguishable from non-irradiated milk. The rennetability of the milk irradiated by the method of Scholl was increased by 30–70 per cent. when compared with unheated milk or with milk irradiated by the method of Hoffmann. It was found that the Scholl lamp had an ultra violet intensity 5700 times greater than that of the Hoffmann lamp. S. K. KON

M. E. F. CRAWFORD, J. GOLDING, E. O. V. PERRY and S. S. ZILVA. **The fat-soluble vitamins of milk.** *Biochem. J.* **24**, iii, 682–91, 1930.

Vitamins A and D were determined in whole milk, in butter churned from the cream separated from the original milk, and in the skim milk (0·1 per cent. fat) to which the buttermilk was added in equivalent quantities. The whole milk, skim milk, and buttermilk had been dried under reduced pressure at a temperature never above 36° C. The growth obtained with skim milk plus buttermilk was proportional to the amount of residual fat present in the doses the animals received, and agreed with the growth of those receiving butter containing corresponding amounts of butterfat. The growth with whole milk was proportional to the milkfat content of the doses. The results suggested that the entire vitamin A and vitamin D content of whole milk and skim milk is associated with the milk fat, and that there is no significant loss of either vitamin due to separating or churning. S. J. ROWLAND

H. HENTSCHEL and O. BACHMANN. **Determination of total sterol and ergosterol in cow's milk. Comparative values for milk of different origin.** *Z. ges. exp. Med.* **71**, 744–54. (*Brit. Chem. Abst.* A, p. 508, April 1931.)

Potassium hydroxide is added to milk, and the fat extracted with ether; after saponification, the unsaponifiable fraction is extracted with ether and treated with digitonin solution. The factor is 0·2431. The following sterol contents of milk (mg.

per litre) are recorded: Rosenheim 77, Allgäu 57, Berlin 60, Central Germany 101. Corresponding values for inactive ergosterol were: 0·19, 0·13, 0·10, —. The spectrum of the fourth sample suggested the presence of activated ergosterol.

W. Weitzel. **Die Vitamine der Milch und ihr Verhalten chemischen, physikalischen und thermischen Einflüssen gegenüber.** (Vitamins of milk and their reaction to chemical, physical and thermic influences.) *Münch. tierärztl. Wochr.* **81**, 104–7, 1930. (*Zbl. f. Bakt.* I, Ref. 99, xxi–xxii, 527, November 1930.)

H. Wieland and T. F. Macrae. **Mechanism of oxidation processes. XXVI. Dehydrogenating enzymes of milk.** *Annalen*, **483**, 217–250, 1930. (*Brit. Chem. Abst.* A, p. 389, March 1931.)

On keeping or by agitation, the power of milk samples to promote the oxidation of xanthine by methylene blue is increased. This is explained by the fact that the union of the fat droplets causes a decrease of the surface on which the xanthine dehydrogenase is probably absorbed. The aldehyde dehydrogenase shows less marked increase in activity on keeping or by agitation. The conclusion is reached that the xanthine and aldehyde dehydrogenases are separate enzymes. Removal of xanthine dehydrogenase from whey can be effected by absorption on calcium carbonate leaving the aldehyde dehydrogenase in solution in a less active form. Hydrogen peroxide inhibits aldehyde oxidation in the presence of methylene blue. The enzyme solutions are able to effect the dismutation of aldehyde (aromatic and aliphatic) into acid and alcohol (optimum *p*H 8·3). The same enzyme is responsible for the aerobic and anaerobic oxidation in the presence of methylene blue and for the dismutation. The effect of cyanide on the three reactions is of the same order of activity.

W. L. Davies

S. Sasaki. **Ueber die serologische Differenzierung der Milch- und Serum-Eiweisskörper.** (Milk and serum Proteins.) *Arb. Med. Univ. Okayama*, **1**, 550–81, 1930. (*Physiol. Abst.* **15**, 10, January 1931.)

Milk globulin and serum globulin are identical proteins which are formed in the maternal blood stream. Casein is first formed in the milk. Milk albumin and serum albumin are different from one another. Milk albumin as well as casein is first formed in the mammary gland. There is a 100 : 40 relationship between the proteins of cow's and goat's milk. This relationship is approximately the same for the casein, albumin and globulin. The formation of the antibodies for the individual proteins is more or less divergent. The formation of that for casein is the most rapid, and in proportion that for albumin the slowest. The specificity of heated casein was confirmed by the precipitin method. Casein is the only heat-stable protein, the others being all heat labile.

J. Zayowsky and P. Allksejeff. **Die Katalase der Milch. I. Die Bestimmung der Milchkatalase mit $KMnO_4$-Titration.** (The catalase of milk. I. The estimation of catalase in milk by permanganate titration.) *Fermentforsch.* **12**, i, 55–66, 1930.

The usual methods of determining catalase in milk by actual measurement of oxygen liberated from hydrogen peroxide have the disadvantage of not being able to demonstrate fine differences and of giving varying results in duplicates. A finer method whereby the undecomposed peroxide is determined by permanganate titration, is described. Two ml. milk and 98 ml. water, 5 ml. 0·3 per cent. H_2O_2 and 5 ml. 10 per cent. H_2SO_4 are mixed together. A control mixture containing 2 ml. of boiled milk is also made. After allowing to stand 30 minutes at room temperature, the excess peroxide is determined by titration with standard decinormal permanganate. This

method gives a more accurate value for the number of milligrams of oxygen liberated from hydrogen peroxide by 100 ml. of milk, but the values are of the same order as those found by Henkit's and Lobeck's methods. W. L. DAVIES

R. A. MORTON and I. M. HEILBRON. **Vitamin A of butter.** *Biochem. J.* **24**, iv, 870–3, 1930.

As the physiological action of the vitamin A of liver oils can be closely simulated by the plant pigment carotene, the non-saponifiable matter from butter was examined spectroscopically for vitamin A and carotene. It was shown to contain both of them, and their potency estimated with some degree of accuracy.

S. J. ROWLAND

PHYSIOLOGY.

J. H. NORRIS. **A chemical investigation in Victoria (Australia) of the blood of cattle and sheep.** *Austr. J. Exp. Biol. and Med.* **7**, iii–iv, 1930. (*Physiol. Abst.* **16**, i, 21, April 1931.)

For steers the average total phosphorus content of the blood in mg. per 100 c.c. was found to be 10·13, for cows 12·59. The average iron content in mg. per 100 c.c. was 60·54 for steers and 57·92 for cows.

A. WESTERLUND. **A contribution to the knowledge of calcium metabolism. III. The relative faecal outgo of calcium in dairy cows.** *Skand. Arch. Physiol.* **60**, 251–63, 1930. (*Chem. Abst.* **25**, iv, 735, February 20, 1931.)

A statistical study of the co-variations of the factors affecting the relative Ca elimination points to the conclusion that the difficulty of keeping lactating cows in Ca equilibrium on the usual feeds is chiefly due to the unsuitability of such controlling factors as the scanty supply of some dietary constituents necessary to guarantee the animal's maximum utilisation of the Ca, *i.e.* Ca itself, as well as P, fat, Na and silage, while such constituents as Mg or Si are supplied too abundantly in proportion to the Ca consumed. Besides, the milk secretion is also of importance, the more copious its flow the smaller the relative faecal outgo of Ca.

J. WATRIN and P. FLORENTIN. **Tumeur utérine et sécrétion lactée chez la lapine.** (Uterine tumour and lactic secretion in the doe rabbit.) *C.R. Soc. Biol.* **104**, xxvi, 1286–8, September 20, 1930.

The authors made a complete examination of a doe rabbit in which abundant milk secretion occurred without pregnancy. They found a uterine tumour which had been caused by retention of the maternal placenta from the previous pregnancy, but none of the foetal placenta; a development of the interstitial tissue of the ovaries which showed no corpora lutea, and other changes (in thyroid and adrenals), which are normally associated with pregnancy. They therefore exclude the foetus, ovary or corpus luteum as the cause of milk secretion and conclude that it was due to the presence of uterine deciduoma in the maternal organism. The authors discuss the possible mode of action of the deciduoma. G. L. PESKETT

G. W. CORNER. **The hormonal control of lactation. I. Non-effect of the corpus luteum. II. Positive action of extracts of the hypophysis.** *Amer. J. Physiol.* **95**, 43–55, 1930. (*Physiol. Abst.* **15**, xi, 667, February 1931.)

The mammary gland of non-pregnant spayed rabbits could not be made to proliferate beyond the normal pubertal degree of the development by either the administration of corpus luteum extract or by experimental production of continuous

corpora lutea in the ovaries. But whole sheep's hypophysis given to spayed virgin rabbits did cause the proliferation of the mammary gland and lactation resembling a full-term gestation. No previous sensitisation by corpus luteum was necessary.

H. H. Cole. **A study of the mucosa of the genital tract of the cow, with special reference to cyclic changes.** *Amer. J. Anat.* **46**, 261–302, 1930. (*Physiol. Abst.* **15**, xi, 668, February 1931.)

The character of the vaginal epithelium normally alters in passing from the vestibule to the cervix. Changes are traced in the vestibule, vagina and cervix from pro-oestrum to post-oestrum. In the vagina proper during the pro-oestrum the surface epithelium consists of large, wide, mucus-secreting cells beneath which are two or three layers of polyhedral epithelial cells. At the time of heat the surface cells are tall and columnar, and the underlying layer is reduced to one or two layers. At 2 days post-oestrum the vaginal epithelium is restored to several layers, and the surface layer is only slightly differentiated from the underlying layers. At 10 days post-oestrum the epithelium is vacuolated and somewhat degenerate. The stroma becomes oedematous during pro-oestrum and oestrum, and the blood vessels are congested. Changes in the cervix closely resemble those in the vagina.

O. Laxa. **Retained milk.** *Ann. Falsificat.* **23**, 609–10, 1930. (*Analyst*, **56**, 660, 184, March 1931.)

Twelve cows in the month of August travelled by rail for 3 days, and were not milked until they arrived at the farm, and four of these then only yielded about 100 c.c. of milk. This milk resembled colostrum, and was white with a brown tint, and had a smell of burnt milk, attributed to the decomposition of the milk sugar. The milk contained water, 78·4; fat, 10·15; casein, 2·65; albumin and globulin, 0·0; albumoses and peptones, 2·92; amino acids, 1·78; milk sugar, 1·50; ash, 1·10; and undetermined substances 1·50 per cent.

J. McFadyean. **The corpora amylacea of the mammary gland of the cow.** *J. Comp. Path. and Therap.* **43**, iv, 291–300, December 1930.

The paper opens with a summary of the more important articles that have already appeared on the subject. These have shown that amyloid bodies occur very commonly in the udders of cows, but that there is no evidence to suggest that they are constantly present. Most of the bodies are not composed of the same material throughout, though all have a stratified structure. The use of the adjective amyloid seems unjustifiable, as no part of them gives the characteristic reactions with methyl violet, or iodine.

The author inclines to the view that the majority of the bodies are formed *ab initio* free in the alveoli from a central portion, which has basophil-staining reactions and is not stratified. Stratification occurs by accretion of materials from the surrounding fluid, the cells of the alveoli do not necessarily play any part in the formation of the bodies. The need for further research work is emphasised. G. L. Peskett

G. N. Porovski. **Effect of milk feeding on secretion of gastric juice.** *Russ. J. Physiol.* **12**, 331–47, 1930. (*Brit. Chem. Abst.* A, p. 1466, November 1930.)

From experiments with dogs with a stomach pouch it is concluded that milk contains a substance stimulating the secretory activity of the gastric glands. The substance is thermostabile, and is soluble in 96 per cent. alcohol, water, and ether, but not in absolute alcohol.

S. V. Telfer. **Mineral metabolism in infancy. II. Utilisation of mineral elements in human milk.** *Glasgow Med. J.* **33**, 10–20, 1930. (*Brit. Chem. Abst.* A, p. 1615, December 1930.)

All of the elements are retained by the infant in the proportion in which they are present in the milk, the retention being approximately 60 per cent. of the intake.

DAIRY HUSBANDRY.

Feeding value of cottonseed hulls as a roughage for growing dairy heifers. The use of cottonseed meal and hulls as a ration for lactating cows. *Texas Sta. Rpt.* p. 857, 1929. (*Exp. Sta. Record*, **63**, v, 472, October 1930.)

The average score of 24 heifers fed cane hay was 80·67 ± 1·01 per cent. in November and for 17 head of the same heifers 76·41 ± 1·45 per cent. in June. The corresponding scores for heifers fed cottonseed hulls were 77·00 ± 1·12 per cent. (22 head) and 72·24 ± 1·91 per cent. (17 head).

No material differences in the general health and condition of the udders of the cows in this test were observed that could be attributed to differences in the rations. Observations on fertility and reproduction did not show that the feeding of cottonseed meal, even in excess, had any significant effects as compared with a balanced ration. Cottonseed meal had no apparent constipating effect as judged by the consistency of the faeces.

E. Edin and G. Sundelin. **Undersökningar angaende fodermärgkal, dess odling och odlingsvärde.** (Investigations on marrow stem kale, its cultivation and feeding value.) *Sweden Cent. Agric. Exp. Sta.* Medd. 376, 1930.

Very useful information on marrow stem kale is supplied in this report, which contains a four page English summary. The notes include the composition, results of digestibility trials and some helpful details regarding the use of the crop for stock feeding.

The average composition of the organic matter was found to be: crude protein 16·4 per cent., fat 3·1 per cent., fibre 18·5 per cent., N-free extract 62·0 per cent. (true protein 10·9 per cent.).

The average digestibility as determined by the chromium oxide indicator was as follows:

	Digestibility %	
	Fresh but last harvest's kale	Dried kale
Crude protein	75	68
Fat	66	25
Fibre	48	53
N-free extract	88	77
Carbohydrates	81	70
Organic matter	78	66
True protein	67	53
True protein with pepsin HCl	83	67

S. Bartlett

W. B. Ellet, C. W. Holdaway, J. F. Eheart and L. D. Lasting. **Feeding hevea rubber-seed meal for milk production.** *Virginia Agric. Exp. Sta. Tech. Bull.* No. **41**, April 1930.

Hevea rubber-seed meal is a new high protein concentrate in the United States. It is the by-product from the kernels of the seed of the Para rubber tree (*Hevea brasiliensis*) after the extraction of the oil. The by-product comes as cake from the

pulp presses and when ground forms a dry, friable and rather fine meal having a pleasant odour. Experiments with this meal have been conducted with dairy cows to determine its value for milk production and the digestibility of its constituents.

The results indicate that Hevea rubber-seed meal may be used as a medium protein concentrate in rations for cows. It is palatable, neither laxative nor constipating in its physiological effect on the digestive system and is apparently equal to linseed meal (equivalent to English linseed cake or cake meal) for milk production. Up to 5 lb. per head daily may be given. The analysis and digestibility coefficients (in brackets) were as follows: moisture, 9 per cent.; crude protein, 27·5 per cent. (71); ether extract, 9·5 per cent. (92); crude fibre, 15·0 per cent. (20); nitrogen-free extract, 33·5 per cent. (58); ash, 5·4 per cent. J. MACKINTOSH

O. E. REED and C. F. HUFFMAN. **The results of a five-year mineral feeding investigation with dairy cattle.** *Michigan Agric. Exp. Sta. Tech. Bull.* No. **105**, February 1930.

In this experiment the effects of a basal ration low in minerals (especially calcium), of mineral supplements in the form of bone flour, limestone rock (finely ground), raw rock phosphate (finely ground), and a complex mineral mixture and of alfalfa hay in place of timothy hay, were studied. Seven lots of five heifers (all Holstein Friesians of similar age and breeding) were used and maintained under this experiment from the age of 6 months to 5 years. Data were obtained on the rate of growth, reproduction, health of cows and calves, milk and fat yields and the effect of the various supplements on appetite and on the bones and teeth. Numerous illustrations of the cows at various ages and of their calves are given and interesting information is supplied on the rate of growth, breeding records, etc.

The results indicate that the general need for mineral supplements for dairy cattle under normal conditions may be greatly exaggerated. Mineral supplements supplying calcium are not greatly needed even when such feeds as timothy hay and other low calcium roughages are used. Raw rock phosphate to the extent of 1·5 per cent. of the grain mixture injured the health and teeth of the animals perhaps by reason of its fluorine content, also the complex mineral mixture used, in the course of time caused harmful effects and injury to the teeth. J. MACKINTOSH

K. W. D. CAMPBELL. **Milking at three eight-hour intervals as a means of investigating variations in the fat and solids-not-fat.** *J. Agric. Sci.* **20**, ii, 213–32, April 1930.

The object of this investigation was to collect evidence on the effect of thrice-daily milking on the yield of individual cows, and on the effect of milking at three eight-hour intervals on the percentage and weight of butterfat. Twelve cows in the herd at the University of Reading Farm were milked thrice daily, at the hours of 6 a.m., 2 p.m. and 10 p.m. throughout a complete lactation; at each milking the milk yielded by each cow was weighed, sampled and the percentages of fat and solids-not-fat determined. The cows were all home-bred Dairy Shorthorns which calved between November 29, 1927, and May 3, 1928, and of different ages, viz. 1st calf 3; 2nd calf 3; 3rd calf 2; 4th calf 2, and 5th calf 2. The management was on uniform lines throughout the period.

It was found that the nine cows increased in milk yield when milked thrice daily by an average of 19·3 per cent., the individual yields showing a range of from a decrease of 21·1 per cent. to an increase of 41·8 per cent. The calculated increase obtained from the three first-calf heifers was 5·6 per cent. These comparisons are made on the milk yields after standardisation by the methods suggested by Sanders. The effects of intervals of varying length between the milkings on the milk yield and percentage of fat is discussed. Tables are given showing the variation in milk yield and fat percentage from day to day. The author suggests that some factor operates at

night tending to lower the rate of fat secretion and that this factor operates whether the night interval be long or short.

[No reference is made in the text to the solids-not-fat content of the milk, and the possibility of the variations in milk yield and fat content being due to changes in the milkers or in the efficiency of milking is not considered.] J. MACKINTOSH

R. E. GLOVER. **Tuberculin testing of graded herds of cows.** *Brit. J. Tub.* **24**, 75–8, 1930. (*Bull. Hyg.* **5**, xi, 883, November 1930.)

Two tuberculin tests are discussed, *i.e.* the subcutaneous and the double intradermal. The advantages and defects of the subcutaneous test are discussed, particularly the desensitisation which may result when the animals are tested at periodical intervals. The factor has led to so-called tubercle free herds not being always free from tuberculosis although all were non-reactors. The double intradermal method, after a period of 5 years' testing has been shown to be much superior to the old method, while it is not followed by desensitisation. The second inoculation after the preliminary sensitising dose is a most important modification, as from 5 to 40 per cent. of reacting animals produce either a negative or doubtful reaction with the first dose. Tuberculins of known potency must be used.

DAIRY INDUSTRY.

United States of America—White House Conference on Child Health and Protection. Report of committee on milk production and control. *U.S.A. Public. Health Rep.* **46**, xiv, 769–811, April 3, 1931.

The report is divided into four parts dealing with: (1) Communicable diseases transmitted through milk; (2) Public health supervision of milk; (3) Nutritional aspects of milk; (4) Economic aspects of milk. Each part has been prepared by a special sub-committee.

(1) Milk-borne epidemics of diphtheria, dysentery, gastroenteritis, paratyphoid, poliomyelitis, scarlet fever, septic sore throat, typhoid fever and undulant fever reported in the U.S.A. during the six years 1924–9 are tabulated and discussed. A special table sets forth the sources of milk infection. The following recommendations are made: I. That pasteurisation be required whenever practicable. II. That pasteurisation is not intended to take the place of the sanitary production of clean and wholesome milk, but rather to provide the final factor of safety from milk-borne diseases. III. That co-operative effort between producers, control and educational officials gives promise of the best and most lasting results.

(2) The following points are discussed: (*a*) The essential elements of the Public Health supervision of milk supplies which include inspection of farms and plants, supervision of the physical examination and testing of cows, laboratory examination of milk, physical examination of workers and residents at farms and plants including laboratory examination of body discharges and pasteurisation control of general market milk. (*b*) The fundamental items which should be included in the laws and regulations relating to the Public Health supervision of milk supplies and the agencies which should enforce them. (*c*) The evaluation of the results of the enforcement of laws and regulations. (*d*) The present status of the Public Health supervision of milk supplies in the U.S.A. (*e*) Recommendations for the White House Conference with reference to future improvements of the supervision of milk supplies in the U.S.A.

(3) The various constituents of milk are enumerated and discussed and recommendations for future research work on milk are made. They include a study of the toughness of the curd formed by rennet coagulation of the vitamins and inorganic

constituents of milk, of the biological value of milk proteins, of the value of different kinds of milk in infant feeding and of the optimal proportion of milk in the diet of infants and children. Further work on colostrum, a detailed study of human milk, and standardisation of equipment for pasteurising milk and researches on the chemistry of casein and the nutritional value of whey and whey powder are considered necessary. The increased production of milk powders, evaporated milk and condensed milk is advocated for reasons of economy.

(4) The following points are discussed: (*a*) The consumption of fluid milk and cream. (*b*) The consumption of other milk products. (*c*) The production of milk. (*d*) The marketing of milk. (*e*) The transportation, processing and delivery. (*f*) The sanitary quality of milk and cream, and suggestions relating to these points are made.

S. K. Kon

F. A. Clark. **Results of the operation of the standard milk ordinance in Missouri.** *Pub. Health Rep.* **46**, xxiv, 1413–24, June 12, 1931.

W. Barton. **Graded milk movement in Scotland.** *Scottish Farmer*, **39**, No. **1983**, 80–1, January 17, 1931.

J. F. Blackshaw and R. Stenhouse Williams. **La production du lait propre en Angleterre.** (Clean milk production in England.) Paper at Comm. d'Étude Fédération Int. Laiterie, 1930. *J. Agric. Belgique*, **12**, xlv, 353–4, November 8, 1930; xlvi, 365–6, November 15, 1930; xlvii, 371–2, November 22, 1930; xlviii, 381–2, November 29, 1930.

J. Berko. **A tej megitelese reduktase es erjesztöproba utjan nyert vizsgalati adatok, valamint sorozatosan vegzett savokmeghataroz sok alapjan.** (Judging milk on the basis of reductase, fermentation and acidity tests.) *Mezogazd. Kutat.* **2**, ix, 417–21, 1929. (*Biol. Abst.* **4**, xi, 2746, November 1930.)

In comparison of the keeping quality of dairy milk and of gathered farmers' milk the reductase and fermentation tests showed that the methods of production and therefore the keeping quality of the dairy milk are much better than those of gathered farm milk. The dairy milk boiled without coagulation after 7 hours, this was not true of the farmers' milk after 2 hours.

K. Diernhofer. **Untersuchungen über den Geschmacksfehler der "schmirgeligen Milch."** (Faulty flavour of "emery" tainted milk.) *Milchw. Forsch.* **10**, v–vi, 319–35, October 1930.

A taint resembling a metallic emery taste can be produced in milk by inoculation with an anaerobic organism of the Fraenkel bacillus type. This organism can be isolated from commercial samples of milk which possess the emery taint. The taint originates from the breakdown of oleic acid and not from butyric fermentation, the development being catalysed by the presence of copper and iron salts in traces. An attempt was made to determine the substance responsible for the taint.

W. L. Davies

H. Barkworth. **The influence of the fat content on the keeping quality of milk.** *J. Min. Agric.* **37**, viii, 803–6, November 1930.

The opinion prevalent in the dairy industry that rich milk does not keep so well as milk of average quality has been studied by classifying 4002 samples of milk of which the bacterial content and keeping qualities were known, into three groups—viz. under 3·0 per cent., between 3·0 and 4·0 per cent. and over 4·0 per cent. It was found that the keeping qualities of all three classes were equally good where the bacterial count was low, but with counts of over 100,000 per c.c. there was a tendency

for the samples with a fat content of over 4·0 per cent. to lose a little in keeping qualities as the count increased. The loss, as shown by this study, amounted to only 2–3 hours in the case of milk with a keeping quality of 42 to 48 hours, and is scarcely of commercial importance. (Had it been possible to include a sufficient number of samples containing 5 per cent. fat more useful information would have been obtained on the keeping qualities of rich milk.) J. MACKINTOSH

G. J. and A. M. HUCKER. **Commercially prepared infant foods.** *New York Bull.* **584**. October 1930.

This is a non-technical bulletin describing the various classes of commercial infant foods (reconstructed infant food, milk modifiers, protein milk, lactic acid milk, powdered, evaporated, and condensed milk). Every infant food should meet the following standards: I. It should be produced under clean conditions. II. There should be no harmful bacteria present. III. The number of bacteria present should be reduced to a minimum. Practically all of the infant foods on the market at the present time meet these standards. S. K. KON

L. A. ALLEN and J. BELL. **The role of milk constituents in bread making.** *J. Roy. Tech. Coll.* Glasgow, **2**, 550–63, 1931.

The paper briefly reviews some of the literature on this subject and mentions the beneficial results following heat treatment of milk or separated milk designed for use in bread making. "Grewe and Holm found that dried skim milk which had been preheated to 73, 83, 93 or 100° C. gave a decided improvement in loaf volume and fermentation tolerance as compared with milks preheated to 50° or 63° C."

In their preliminary experimental work the authors attempted to compare the relative values of whole milk, separated milk, whey and whey from which the albumen had been precipitated; thus removing in turn the fat, the fat and casein, and lastly the fat, casein and albumen.

In these experiments five batches of 2 lb. loaves (four in each batch), were made in order to compare colour, texture, flavour, etc. The effect on the keeping quality and water content of the loaves does not appear to have been determined.

The baking formula selected was as follows: 4 lb. strong wheat flour, $1\frac{1}{4}$ lb. soft winter wheat flour, $\frac{1}{2}$ oz. sugar, $\frac{1}{2}$ oz. lard, $1\frac{1}{2}$ oz. salt, 2 oz. yeast and 1500 c.c. water. The fermentation period was 75 minutes, the proof period 30 minutes, and the time of baking 30 minutes.

The results were as follows:

Texture. Whole milk, separated milk and whey, first, protein free milk next, and control last.

Colour. Whole milk first, separated milk and whey second, control third, and protein free milk last.

Flavour. Whole milk, separated milk and whey first, followed by control bread, the protein free being put last.

The results were confirmed by a series of batches of 1 lb. loaves made on the same lines.

Much of the practical work was devoted to an estimation of the effect of milk constituents on the volume increase of fermenting dough.

The method consisted in measuring the increase in volume taking place in dough contained in a measuring cylinder which was placed in a water bath at a constant temperature. For this purpose a mixture of strong spring wheat flour 225 g., yeast 22·5 g., salt 9 g., water 250 c.c. was used. These constituents were first well mixed with a pestle and mortar and the mixture was then transferred to a mechanical whisk where it was agitated for exactly 120 seconds. A weighed quantity was transferred to a 500 c.c. measuring cylinder.

The cylinders were maintained at 29° C. and the increase in volume noted which took place between the commencement of fermentation and the point where the risen dough broke and started to decrease in volume.

Four determinations were made with each type of milk or milk product and the average taken. It is claimed that this method gave more concordant results than the customary methods of determining the volume of the finished loaf. The extent to which the indirect method was a true index of the actual loaf volume is not stated owing to the difficulties experienced by the authors in recording small differences by the customary methods.

The effect of milk constituents on the rate of evolution of gas in fermenting dough was also determined. From this work the authors drew the following conclusions:

1. Addition of either whole milk, separated milk or whey to the dough used for bread making resulted in improved texture, colour and flavour in the finished loaf. As regards colour, whole milk was found to be superior to the other two products.

2. Experiments conducted with separated milk, whey, and whey from which the albumen had been extracted, respectively, in order to determine their influence on the volume increase in a fermenting dough, showed that separated milk had a slightly detrimental effect, whey had an appreciably detrimental effect, while whey minus albumen had an appreciably beneficial effect.

3. The theory that the albumen is a determining factor in volume increase was confirmed by some experiments made with a suspension of egg albumen, when it was found that the increase in a fermenting dough was smaller than that in a control dough containing no albumen.

4. Boiling the milk products for 15 minutes under reflux prior to their use in a dough mix was found to have a remarkably beneficial effect on the volume increase during fermentation. It is suggested that this is due to the coagulation of the albumen which normally exercises a softening influence on the gluten, thus decreasing its holding power. Even the coagulated albumen was found to have a small detrimental effect.

5. Dried separated milk prepared by the pressure spray system was found to decrease the volume of a fermenting dough, compared with a control dough containing no milk. If the milk was boiled prior to drying, however, this detrimental effect was largely counterbalanced. It is therefore important when using separated milk for bread making to choose a variety which has been preheated, so that the beneficial effect on the texture, flavour, colour and nutritional value may not be counterbalanced by a detrimental effect on the volume.

6. Lactose had no effect on the volume of the dough. Small quantities of lactic acid, on the other hand, resulted in a slight improvement.

7. Whey, boiled whey and whey minus albumen respectively were found to increase the rate of evolution of carbon dioxide from a fermenting dough, while separated milk, raw or boiled, decreased it. It is suggested that the phosphates in the milk tend to increase the rate of fermentation but that the casein has an inhibitory effect. Removal of the casein in the preparation of the whey allowed the beneficial effect of the phosphates full play. Albumen too, appears to have an appreciably damping effect on the fermentation.

8. Rennin was found to have no effect on the rate of fermentation of dough by yeast.

J. GOLDING

C. C. Walts and M. S. Libbert. **Factors influencing the flavour of butter. I. The effect of various starter cultures on the flavour of butter. II. The effect of various neutralisers on the flavour of butter.** *Arkansas Agr. Exp. Sta. Bull.* **249**, February 1930.

I. Nine different commercial starter cultures were compared and only minute differences in the flavour produced were noted by the judges. At the same time control butter made without the addition of a starter scored only 0·49 to 2·3 per cent. less marks than the butter made with the addition of a commercial starter.

II. Eleven neutralising agents for the same cream were used in experimental butter making, but insufficient evidence was available to show that any one agent was better than the others. E. C. G. Maddock

W. H. Udy. **Mottled colour in cheese.** *N.Z. Jour. Agric.* **42**, iv, 244–5, April 1931.

This defect occurring mostly in hot weather, appears as a patchy discoloration approaching complete bleaching in some areas of the annatto colouring of New Zealand coloured cheese. Investigations revealed that the effect of direct sunlight falling on milk and curd in the vat was an important cause of the mottled colour. Five minutes' exposure of milk with annatto in a vat to direct sunlight caused appreciable surface bleaching and 30 minutes caused total bleaching. Sunlight acting through a few inches of whey or whey-soaked curd caused the same partial bleaching effect. Exposure of vats to direct sunlight was common, and mottling was most prevalent in cheese made in these vats. Methods of diffusing sunlight are suggested. W. L. Davies

P. S. Lucas, T. Matsui and O. E. Mook. **Influence of sugar and butterfat on the quality of ice cream.** *Michigan Agric. Exp. Sta.* 1930, Spec. Bull. **201**. (*Brit. Chem. Abst.* B, 134, February 6, 1931.)

Increased fat content within the range 8–14 per cent. improved the flavour, body, texture, and resistance to melting of ice cream. Mixtures with the lower fat content became coarse on storage. Increasing milk solids-not-fat (6–12 per cent.), improved texture and increased the acidity without affecting resistance to melting. With more than 12 per cent. of solids-not-fat the mixtures were "sandy."

REVIEW

Ministry of Agriculture. **Report on the marketing of dairy produce in England and Wales. Part I. Cheese.** *Min. Agric. Economic Series*, No. **22**.

In Part I, the outline of types and varieties of cheese is ably drawn. The cheese-making areas are briefly sketched, and the origin of factory cheese making is discussed. This is followed by a chapter on supply, in which a diagram, setting out the average monthly imports of cheese over a five-year period reveals the remarkably small variation that exists owing to the fact that the exporting seasons from New Zealand and Canada, Great Britain's chief suppliers, only appreciably overlap in July and August. In the chapter on demand there is an interesting table showing the per capita consumption of cheese in various countries. Great Britain with 9·5 lb. per annum occupies a middle position between the extremes of Switzerland with 23·3 lb. and Canada with only 3 lb. per head. The preparation for market, methods of marking and grading cheese are described in detail, and the efforts of the Cheshire and Cheddar cheese farmer producers to grade and mark their cheese receives special attention. A suggested scheme for a National Mark for all varieties of cheese is outlined and its early introduction foreshadowed. The illustrations are admirable, and there is a useful appendix of tabulated information. E. Capstick

STATISTICS

Number of Dairy and Other Cattle.

The following table shows comparative figures of the dairy herd, and of other cattle, in the principal countries within the British Empire in which dairying is of major importance. The figures are in each case the latest available, and are generally of a preliminary nature, but it should be noted that the estimates of livestock relate to different dates in the various countries. It should also be noted that classification of cattle into the dairy herd and other cattle is not a matter of uniform practice throughout the Empire, but the figures give a fairly accurate indication of the extent of the dairy herd and of any change in comparison with the preceding census.

Countries	Year*	Cows and heifers in milk or in calf 000 head	Other cattle 000 head	Total cattle 000 head
England and Wales	1931	2790	3274	6,064
	1930	2715	3135	5,850
Scotland	1931	452	757	1,209
	1930	453	783	1,236
Northern Ireland	1931	259	421	680
	1930	256	417	673
Irish Free State	1931	1223	2819	4,042
	1930	1225	2813	4,038
Canada	1930	3683	5254	8,937
	1929	3685	5140	8,825
Australia	1929–30	Not available		11,202
	1928–29	2467	8834	11,301
New Zealand	1930	1424	2297	3,721
	1929	1371	2075	3,446
Union of South Africa	1929	Not available		10,518†
	1928	Not available		10,478
Kenya	1931	71	166	237‡
	1930	68	159	227

* Estimates relate to June except New Zealand (January 1), South Africa (August 31), Kenya (February 28, 1931 and July 31, 1930); for Australian States estimates refer to December 31 except New South Wales and F.C.T. (June 30 following).

† Including 6,773,000 cattle on farms owned by Europeans.

‡ Relates solely to European owned cattle; the estimated number of native-owned cattle in Kenya was 4,966,000 on July 31, 1930.

Trade in Dairy Produce.

The following tables give details of the trade in butter and cheese of the principal countries handling these products during the first 6 months of 1931, with comparative figures for 1930. The particulars are based on information published by the International Institute of Agriculture, Rome, brought up to date, where necessary, from official sources.

Exports and imports of butter. Six months (January to June).

Countries	Exports		Imports	
	1931	1930	1931	1930
Exporting countries	000 lb.	000 lb.	000 lb.	000 lb.
Austria	1,166	1,786	1,052	293
Denmark	190,579	187,294	972	822
Estonia	12,436	11,378	—	—
Irish Free State ...	12,573	19,703	3,148	2,754
Finland	21,433	22,051	—	—
Hungary	701	1,561	112	—
Latvia	16,945	17,655	18	24
Lithuania... ...	7,319	5,432	—	—
Netherlands ...	39,699	47,792	2,718	1,636
Norway	1,093	229	79	267
Poland	11,903	10,278	20	11
Sweden	23,171	30,931	4	13
U.S.S.R.	18,052	9,833	—	—
Argentina... ...	28,444	25,743	—	—
India	185	309	165	132
Syria and Lebanon	996	1,087	121	37
Australia	93,981	57,367	—	—
New Zealand ...	114,070	122,207	—	—
Importing countries				
Belgium	1,270	1,213	18,805	10,029
Czecho-Slovakia ...	302	448	2,200	293
France	4,594	5,137	28,949	9,279
Germany	143	348	102,586	129,890
Greece	—	—	981*	765*
Italy	1,045	1,323	4,405	1,283
Spain	51	95	33	119
Switzerland ...	4	24	12,207	9,264
United Kingdom...	21,832	7,449	446,460	404,248
Canada	2,366	399	2,813	32,710
U.S. America ...	1,210	1,841	728	1,872
Ceylon	—	—	322	401
Java and Madura	—	—	4,255	4,325
Japan	—	—	128	406
Algeria	24†	24†	1,362†	1,288†
Egypt	15	9	1,199	1,263
Tunis	2	2	476	439

* January 1 to July 31.

† January 1 to April 30 only.

Exports and imports of cheese. Six months (January to June).

Countries	Exports		Imports	
	1931	1930	1931	1930
Exporting countries	000 lb.	000 lb.	000 lb.	000 lb.
Czecho-Slovakia ...	4,094	3,241	1,556	1,365
Denmark	4,575	5,862	313	346
Finland	3,265	1,995	13	15
Italy	44,229	41,998	5,139	6,014
Lithuania... ...	1,060	701	4	4
Netherlands ...	91,192	97,072	604	679
Norway	999	611	300	346
Poland	1,413	1,045	408	538
Switzerland ...	28,724	32,847	3,093	2,275
Yugo-Slavia ...	1,530	1,023	128	152
Canada	7,813	9,961	679	946
Australia	3,228	1,911	15	137
New Zealand ...	112,094	114,561	2	2
Importing countries				
Austria	2,864	1,219	3,294	3,124
Belgium	379	397	23,127	23,321
France	17,227	21,409	38,960	27,902
Germany	2,983	2,471	58,110	61,688
Greece	115*	161*	2,017*	1,634*
Hungary	68	33	163	227
Irish Free State ...	44	57	1,171	1,113
Portugal	—	—	271	340
Spain	148	90	1,750	2,635
Sweden	—	—	778	624
United Kingdom...	3,532	4,383	163,070	169,186
United States ...	959	1,179	30,243	40,583
India	2	2	432	470
Java and Madura	—	—	741	752
Syria and Lebanon	66	97	357	328
Algeria	77†	79†	2,826†	3,948†
Egypt	33	35	3,882	3,556
Tunis	20	7	1,034	871

* January 1 to July 31.

† January 1 to April 30 only.